

## Supporting Information

### **Synthesis of Fluorescent $\alpha$ -Chymotrypsin A-functionalized Gold Nanoclusters and Their Application to Blot-based Technology for Hg<sup>2+</sup> Detection**

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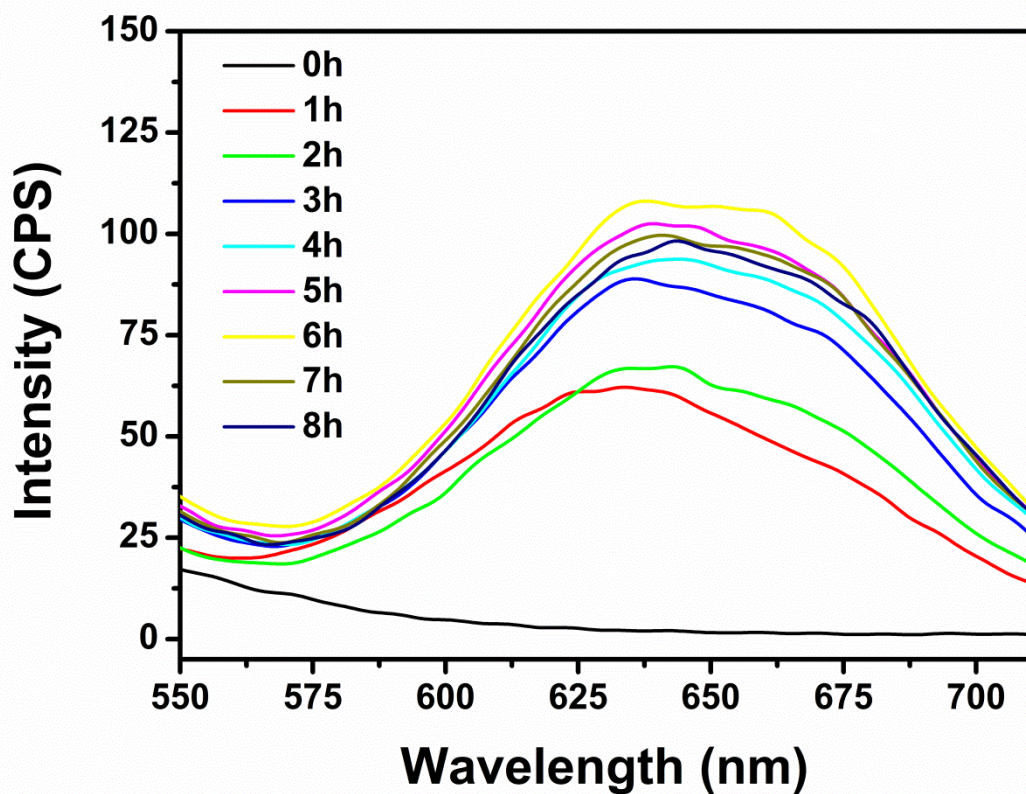
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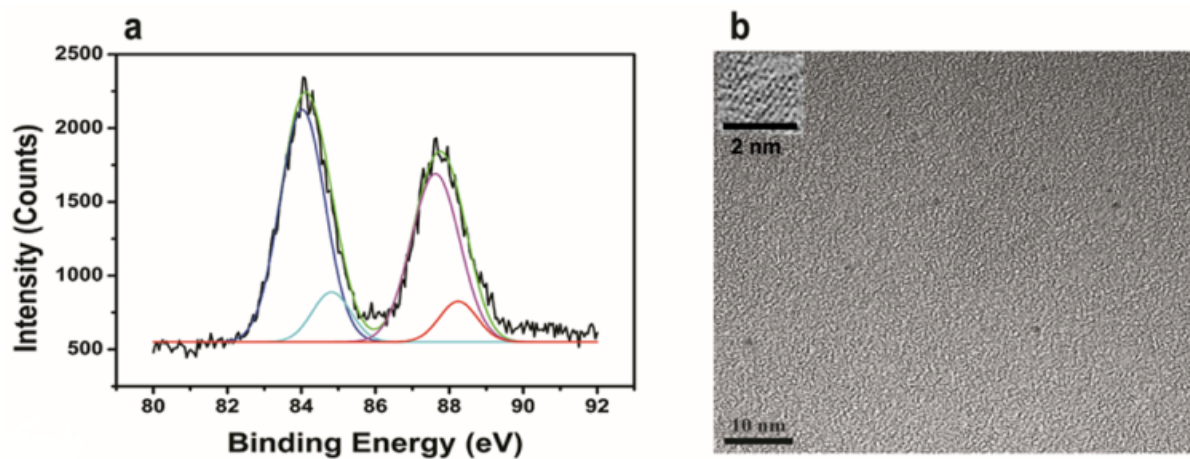
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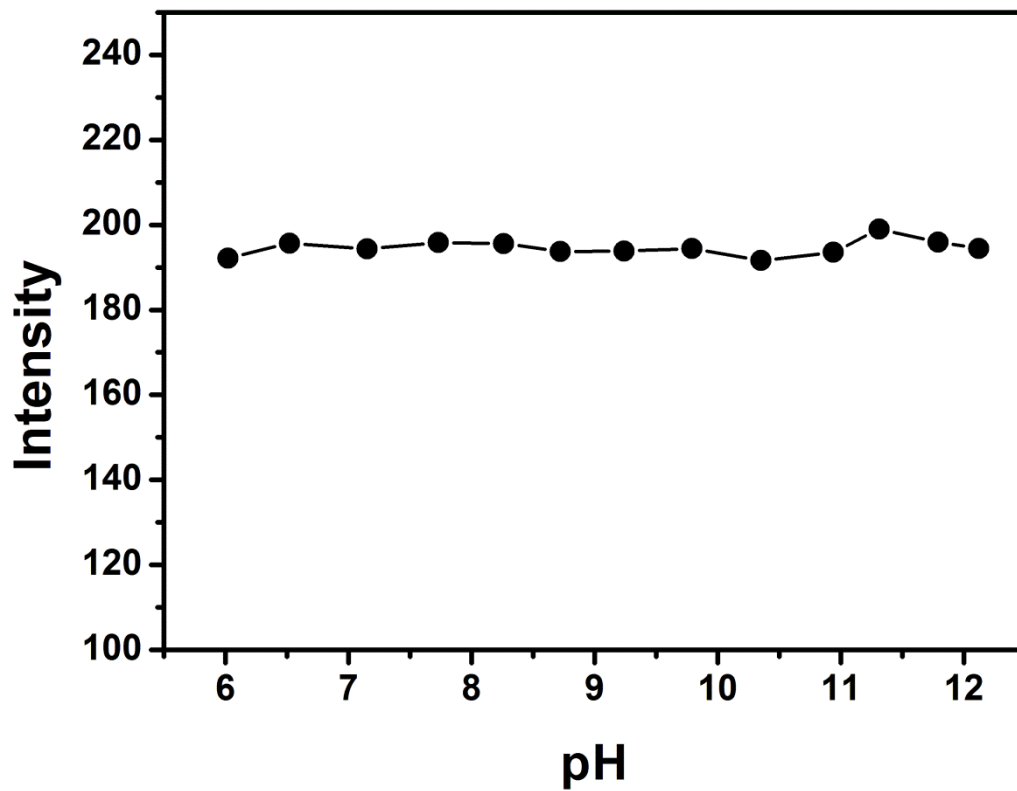
1. **Fig. S1** The fluorescence spectra of synthesized AuNCs@CTRA with different reaction time.
2. **Fig. S2** XPS characterization and TEM image of AuNCs@CTRA.
3. **Fig. S3** The pH stability of the AuNCs@CTRA as indicated by the fluorescence intensity.
4. **Fig. S4** Fluorescence decays of AuNCs@CTRA in HEPES buffer.
5. **Fig. S5** The FT-IR spectra were used for monitoring the synthesis of AuNCs@CTRA.
6. **Fig. S6** The CD spectrum of pure CTRA and AuNCs@CTRA in the aqueous solution.
7. **Fig. S7** The enzymatic activity evaluation of CTRA and CTRA@AuNCs.
8. **Fig. S8** The fluorescence intensity of AuNCs@CTRA in the presence of Hg<sup>2+</sup> or the combination of Hg<sup>2+</sup> and other metal ions.



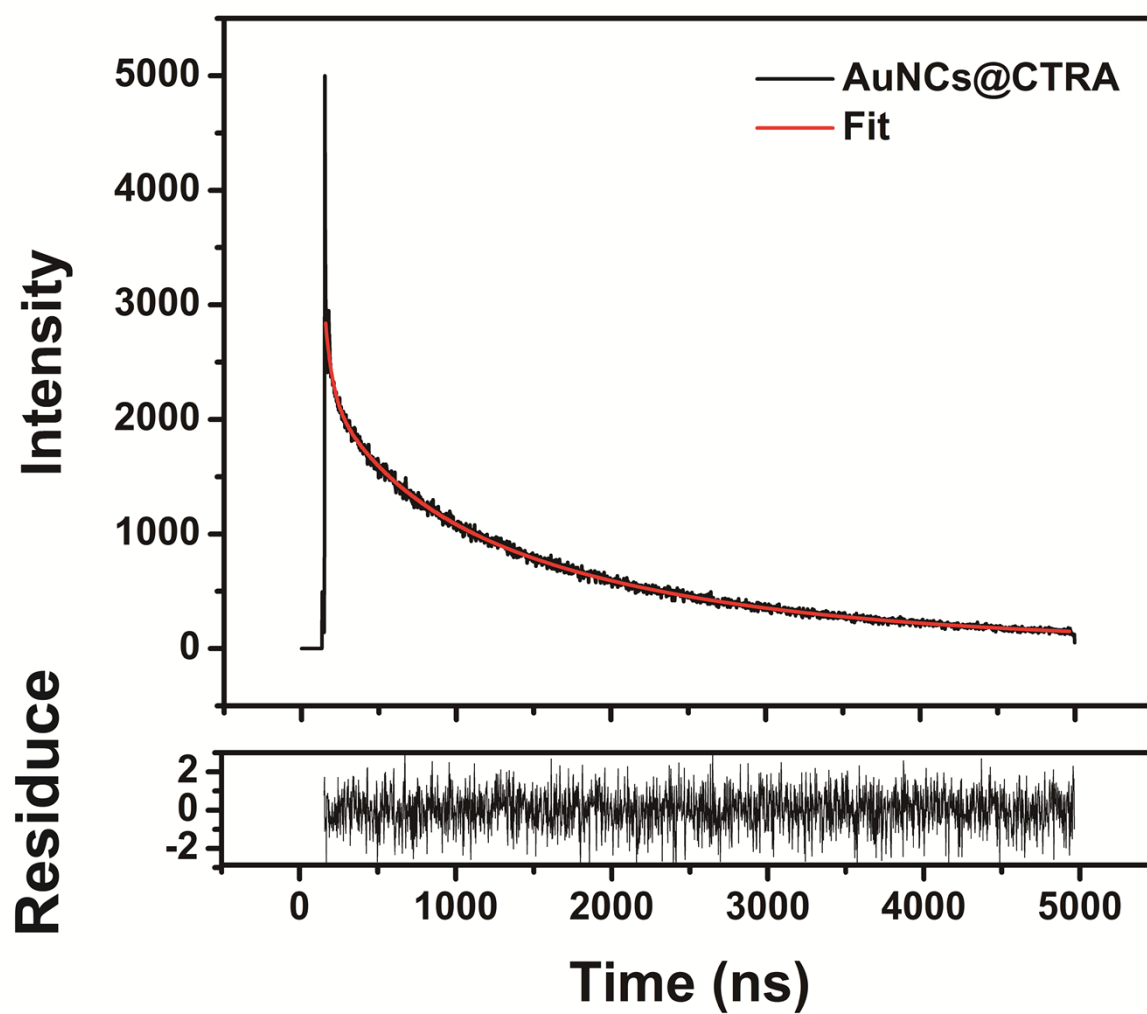
**Fig. S1** The fluorescence spectra of synthesized AuNCs@CTRA with different reaction time (inset).



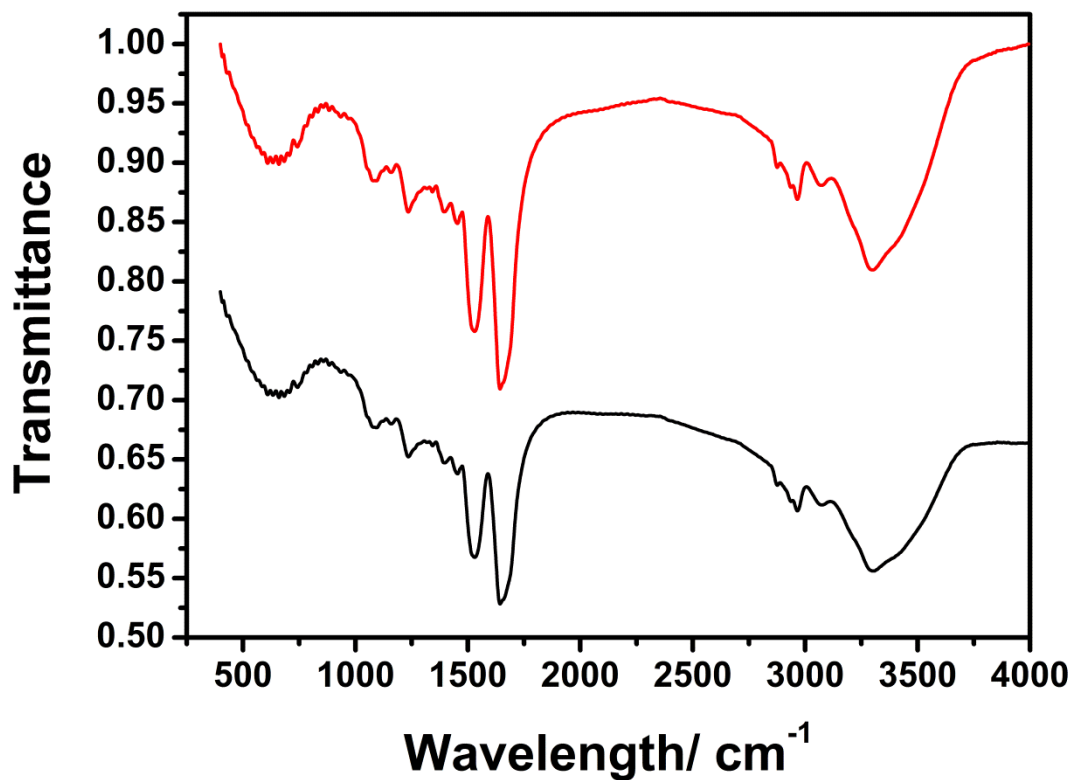
**Fig. S2** XPS characterization and TEM image of AuNCs@CTRA. (a) XPS spectra explain the binding energy of Au4f of AuNCs@CTRA. Navy blue and Light Blue curves indicate Au(0) and Au(I), respectively. (b) TEM image of AuNCs@CTRA, The gold is clearly visible in the TEM image.



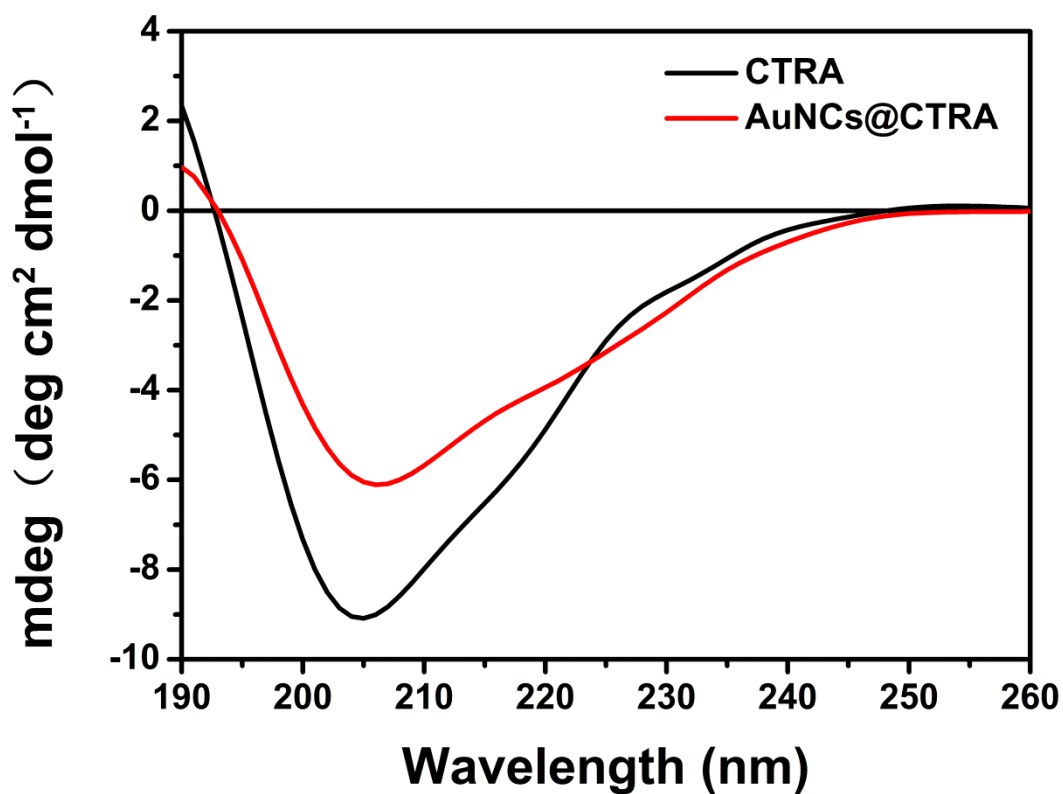
**Fig. S3** The pH stability of the AuNCs@CTRA as indicated by the fluorescence intensity for the CTRA stabilized gold nanoclusters (1.0 mg/mL).



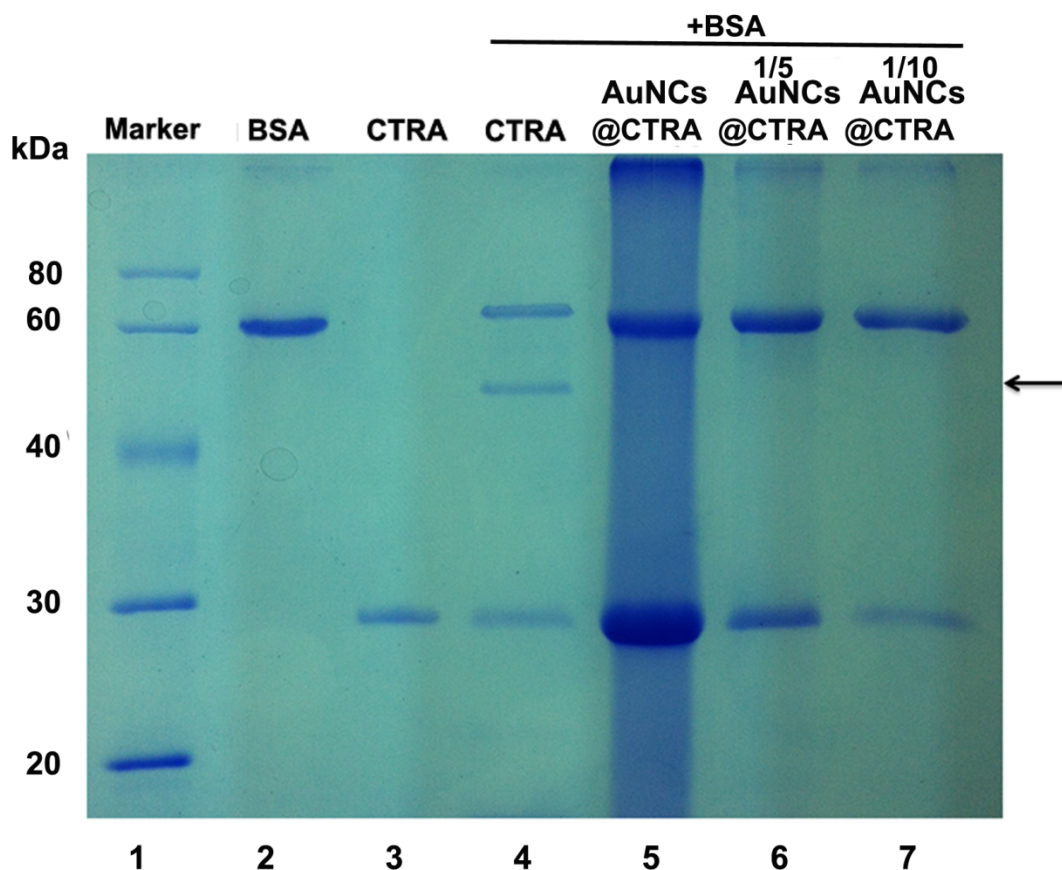
**Fig. S4** Top: Fluorescence decays of AuNCs@CTRA in HEPES buffer (20 mM, pH 7.5). Bottom: Residuals of the fits.



**Fig. S5** The FT-IR spectra were used for monitoring the synthesis of AuNCs@CTRA. The black and red curve depicted the spectrum of pure CTRA and that of AuNCs@CTRA, respectively.

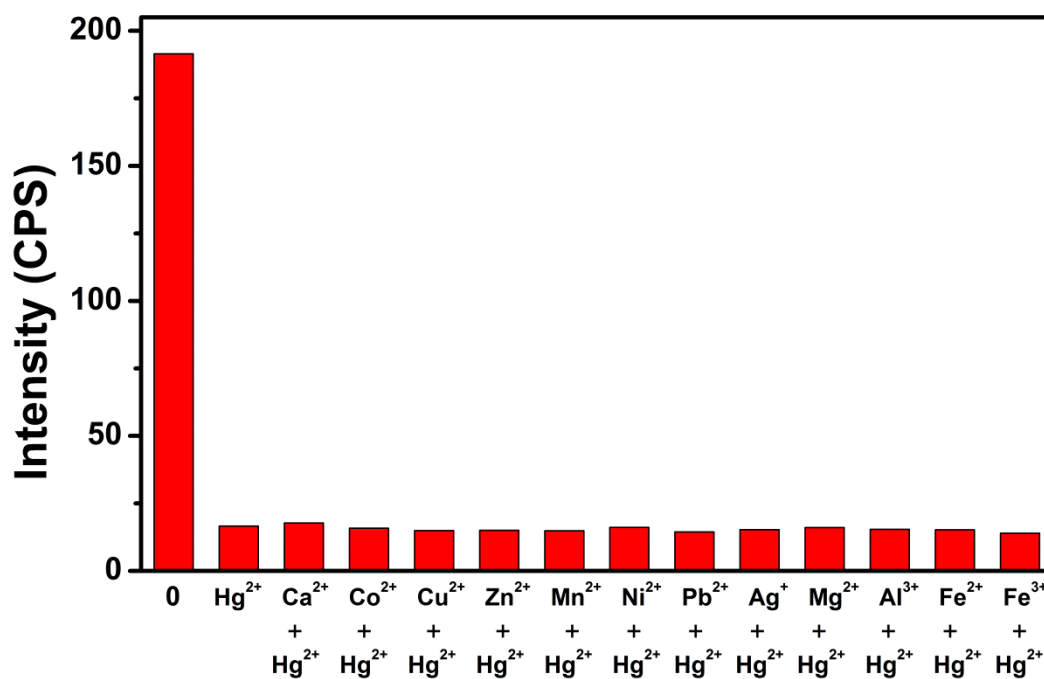


**Fig. S6** The CD spectrum of pure CTRA (Black) and AuNCs@CTRA (Red) in the HEPES buffer (20 mM, pH 7.4).



**Fig. S7** SDS-PAGE showed the activity profile of CTRA and AuNCs@CTRA. The amount of BSA (as substrate, lane 2) and CTRA or AuNCs@CTRA (as enzyme, lane 3-5) were 0.5 and 0.3 mg, respectively. Arrow pointed to the digested BSA product. The SDS-PAGE showed that the CTRA activity was lost during the gold nanoclusters synthesis but the polypeptide chain remained uncleaved.





**Fig. S8** The fluorescence intensity of AuNCs@CTRA (1.0 mg/mL) in HEPES buffer (20 mM, pH 7.5) in the presence of Hg<sup>2+</sup> (10.0 μM) or the combination of Hg<sup>2+</sup> (10.0 μM) and other metal ions (10.0 μM).

**Table S1.** Properties of AuNCs-based methods for the determination of Hg (II)

No.	Detection limit	Readability by naked eye		Reference No.
		under UV light	under visible light	
1	0.6 nM	No	No	[1]
2	80 nM	No	No	[2]
3	25 nM	No	No	[3]
4	50 nM	No	No	[4]
5	50 nM	No	No	[5]
6	50 $\mu$ M	No	Yes	[6]
7	8 nM	Yes	Yes	Proposed method

## References

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