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PCR-free Quantification of Multiple Splice Variants in Cancer Gene by Surface Enhanced Raman Spectroscopy

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Abstract

We demonstrate a surface enhanced Raman spectroscopy (SERS) based array platform to monitor gene expression in cancer cells in a multiplex and quantitative format without amplification steps. A strategy comprising of DNA/RNA hybridization, S1 nuclease digestion, and alkaline hydrolysis was adopted to obtain DNA targets specific to two splice junction variants $\Delta(9, 10)$ and $\Delta(5)$ of the breast cancer susceptibility gene 1 (BRCA1) from MCF-7 and MDA-MB-231 breast cancer cell lines. These two targets were identified simultaneously and their absolute quantities were estimated by a SERS strategy utilizing the inherent plasmon-phonon Raman mode of gold nanoparticle probes as a self-referencing standard to correct for variability in surface enhancement. Results were then validated by reverse transcription PCR (RT-PCR). Our proposed methodology could be expanded to a higher level of multiplexing for quantitative gene expression analysis of any gene without any amplification steps.

Keywords

Quantification; multiplex detection; SERS; splice variants

Introduction

Studying gene expression at the transcript level is a vital and commonly adopted approach in biological and biomedical sciences. Effective and efficient screening of gene expression requires analytical techniques with ability to detect multiple transcripts and provide quantitative estimates of especially the lowly abundant transcripts which are most commonly analyzed qualitatively via PCR amplification. Conventional PCR for gene expression analysis is semi-quantitative in nature. In addition, if more than one pair of primers is used in a PCR reaction to amplify the corresponding segments of target DNA simultaneously, extensive optimization is required. Furthermore, different amplicons are often amplified at different efficiencies to result in competition among multiple primer pairs in the reaction. Although real-time PCR is known for its ability to quantify and multiplex, it is not always effective in situations where high amplification efficiency is not available¹ and challenges do exist in a few technical aspects, such as template quality and data interpretation especially when dealing with very low copy number of targets². In addition, fluorescence-based detection poses a limit on the degree of multiplexing because of the broad and overlapping emission profiles when higher levels of multiplexing are required.^{3,4} The application of other common methods for gene expression studies, such as northern blots and quantitative nuclease protection assays, are either semi-quantitative or lack the needed sensitivity⁵. Microarray is undoubtedly an

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established method for genome-wide analysis. However, such an extensive assay may not always be necessary for many specific applications and is more expensive compared to other techniques. In addition this approach requires a universal genomic data quality control standard for improved reliability.⁶

To meet the requirements of detection sensitivity and multiplexing capability for gene expression studies, surface enhanced Raman spectroscopy (SERS) could be an extremely promising alternative because of its intrinsic sharp fingerprints,^{4,7} single-molecule sensitivity,⁸ and availability of a wide range of photostable labels. Many SERS-based approaches exist for nucleic acid detection. Typical probe design includes adsorption of fluorophore-labeled DNA onto colloidal silver particles,^{9,10} covalent attachment of fluorophore-labeled DNA onto gold nanoparticles,¹¹ the use of plasmonic nanoprobe with metal nanoparticles functionalized with a stem-loop DNA molecule tagged with a Raman label,¹² a surface enhanced resonance Raman scattering (SERRS) beacon approach,¹³ and sequential attachment of DNA strands and nonfluorescent Raman tags to gold nanoparticles.¹⁴ In addition, different substrates and detection platforms have been developed^{11,15,16,17} and multiplex^{11,15,18,19} detection has been demonstrated.

Although successful in qualitative studies, previous SERS applications were limited in providing quantitative information due to the variability of surface enhancement.^{20,21} Ongoing efforts in fabricating tunable and reproducible substrates^{22–25} are aimed at controllable SERS activity, however, uniform SERS for reliable quantification has not been fully achieved. Although quantitative behavior has been shown with a dilution series of dye-labeled DNA sequences,^{10,18,26} no quantitative detection of unknown DNA targets was demonstrated using these systems. An alternative SERS-based quantification strategy using isotope-edited dyes as internal standard was proposed,^{27,28} however, this approach entails the synthesis of new dyes and requires optimization. Hence, a consistent SERS-based quantification method to evaluate unknown nucleic acid targets without amplification will be highly desirable.

In our study, we demonstrate a SERS approach to identify and quantify multiple gene segments extracted from cells based on the concept of using the plasmon-phonon bands as a self-referencing standard.^{29,30} We used the breast cancer susceptibility gene 1 (BRCA1) as a model gene and its two alternative splice variants, $\Delta(9, 10)$ (splice junction skipping exons 9 and 10) and $\Delta(5)$ (splice junction skipping exon 5), as targets. Alternative splicing is a crucial mechanism for generating protein diversity and BRCA1 is a good model because it exhibits cell- and tissue-specific alternative splicing patterns that could serve as a potential cancer marker. We compare the differential expression of these two BRCA1 splice variants ($\Delta(9, 10)$ and $\Delta(5)$) in two breast cancer cell lines, MCF-7 and MDA-MB-231, simultaneously using nonfluorescent Raman probes by isolating RNA from cells, DNA/RNA hybridization, S1 nuclease digestion, alkaline hydrolysis and an array-format SERS sandwich assay. Quantification results were validated by reverse transcription PCR (RT-PCR) and compared with literature, where available. Since the demonstrated SERS method can monitor multiple fragments at a high level of sensitivity without any amplification steps, lowly expressed splice variants could be detected and quantified on an absolute scale. Thus, this method can serve as a complementary detection tool to the existing methods and yet fill the niche in detecting very low quantities accurately and efficiently.

Experimental Methods

Preparation of DNA targets from cancer cells

MCF-7 and MDA-MB-231 cells were grown in Eagle's Minimum Essential Medium (ATCC) supplemented with 0.01 mg/mL bovine insulin and 10% of FetalPlex animal serum complex (Gemini Bio-Products) and ATCC-formulated RPMI-1640 medium supplemented with 10%

of FetalPlex animal serum respectively. Total RNA was first isolated from approximately 10^7 cells using the QIAGEN RNeasy Mini Kit. A 30 μ L of hybridization cocktail (20 μ L of total RNA, 9 μ L of 3 $\times$ aqueous hybridization solution and 1 μ L of DNA oligonucleotides with sequences complementary to the RNA segments of interest) was then added and heated to 75 $^{\circ}$ C for 10 min, and the mixture was incubated overnight at 55 $^{\circ}$ C. Next, 270 μ L of S1 nuclease mix was added and the mixture was incubated at 37 $^{\circ}$ C for 45 min. Hydrolysis solution (1.6 M NaOH and 135 mM EDTA) was then added and the mixture was incubated at 95 $^{\circ}$ C for 15 min. Finally, a neutralizing solution (1 M HEPES, 1.6 M HCl and 6X SSC) was added to suspend the DNA targets of interest in a neutral media.

SERS sandwich assay

Sequences of BRCA1 splice junctions $\Delta(9, 10)$ and $\Delta(5)$ were obtained from NIH maintained GenBank (accession number U14680) as listed in Table 1. SERS sandwich assay was performed on a gold coated glass slide covered with a silicone mask containing an array of wells (3 mm in diameter).¹⁹ In a typical assay, 10 μ L of 1 μ M capturing strand (CS) with the sequence spanning the downstream half of the exon/exon junction and 3' thiol modification was spotted onto the slide and incubated in a humidity chamber at room temperature for 6 h. Then 10 μ L of 1 mM 6-mercapto-1-hexanol was added and incubated overnight in a humidity chamber at room temperature to reduce nonspecific binding. Next, 10 μ L of the target strand (TS) with the sequence complementary to the exon/exon junction was added to hybridize with the immobilized CS by incubating in a humidity chamber at 37 $^{\circ}$ C for 4 h. Finally, 10 μ L of DNA-AuP-RTag probe (synthesis procedure detailed in Sun et al.¹⁴) with probing strands (PS), containing the same sequence as the upstream half of the exon/exon junction with 5' thiol modification, and nonfluorescent Raman tags (RTag) covalently attached to 40 nm gold nanoparticles was hybridized to the overhanging part of TS by incubating in a humidity chamber at 37 $^{\circ}$ C for 4 h. In this study, 4-mercaptopyridine (RTag-1) and 2-thiazoline-2-thiol (RTag-2) were used as nonfluorescent Raman labels. For multiplex detection, sandwich structures formed by different sets of sequences were immobilized on the same detection spots. All tests can be performed on the same slide.

A SENTERRA confocal Raman system (Brucker Optics) was used in this study. A 785 nm diode laser with 10 mW power at the laser source and a 50X air objective (N.A. 0.7, infinity and flat field corrected) were used to acquire all the Raman spectra. A 20 s integration time was chosen. OPUS software was used to analyze all the spectra.

RT-PCR

After RNA was treated with DNase I (New England BioLabs), the iScriptTM cDNA Synthesis Kit (Bio-Rad Laboratories) was used to synthesize cDNA. Then PCR was performed in a DNA Engine (PTC-220) Peltier Thermal Cycler (MJ Research) using *Taq* DNA polymerase (Fisher Scientific) and the corresponding primers. Finally, PCR products were analyzed in 3% of agarose gel prestained with ethidium bromide.

Results and Discussion

To elucidate expression patterns of specific genes in different cell lines, three major tasks were defined: (1) isolation of the nucleotide targets of interest from the isolated RNA pool; (2) qualitative assessment of these targets by SERS; and (3) quantification by SERS and validation by conventional methods. In this study, BRCA1 gene was used as the model gene and absolute expression levels of the two splice junction variants, $\Delta(9, 10)$ and $\Delta(5)$, in the two breast cancer cells lines, MCF-7 and MDA-MB-231, were determined using our SERS platform.

Detailed detection procedures are described in Figure 1. First, a mixture of single-stranded DNA oligonucleotides (40 bp) complementary to exon/exon junctions $\Delta(9, 10)$ and $\Delta(5)$ were respectively added to the isolated total RNA from the corresponding cancer cell line and allowed to hybridize. Then S1 nuclease was added to digest the single-stranded nucleic acids, including unhybridized parts of specific mRNA, all the nonspecific RNA, and excess DNA oligonucleotides, leaving only the specific DNA-RNA duplexes intact, since S1 nuclease can hydrolyze single-stranded RNA or DNA into 5' mononucleotides and remove heteroduplexes containing mismatched regions³¹. Alkaline hydrolysis was subsequently applied to inactivate S1 nuclease and hydrolyze the RNA components of the DNA-RNA duplexes. The resulting DNA target strands (TS) contained sequences specific to $\Delta(9, 10)$ and $\Delta(5)$ in proportion to their mRNA expression. Quantification of these DNA targets provides an estimate of the expression of splice junctions $\Delta(9, 10)$ and $\Delta(5)$ in the corresponding cell line.

Figure 1 shows the sandwich structure formed by linking capturing strand (CS) and probing strand (PS) with the corresponding target strand (TS) via hybridization. When the sandwich structure is formed, Raman signals from the corresponding nonfluorescent Raman tag would be detected. It should be noted that T₁₀ was used as a linker for both CS and PS to minimize nonspecific adsorption of DNA molecules onto the gold surface because thymine has the lowest affinity to gold compared to other nucleotides.³² A layer of 6-mercaptol-1-hexanol molecules was applied after CS immobilization to prevent nonspecific adsorption of DNA from solution and displace nonspecifically adsorbed CS molecules to increase detection specificity. To monitor the $\Delta(9,10)$ and $\Delta(5)$ DNA targets simultaneously, a mixture of capturing strands comprising of sequences complementary to the upstream half of the interested targets and a mixture of DNA-AuP-RTag probes bearing sequences complementary to the downstream half of the interested targets were used for the duplex detection spot. To demonstrate duplex detection of splice variant expression, four cases were investigated: detection of splice junctions in MCF-7 using $\Delta(9, 10)$ -AuP-RTag-1 and $\Delta(5)$ -AuP-RTag-2 probes; detection of splice junctions in MCF-7 using $\Delta(9, 10)$ -AuP-RTag-2 and $\Delta(5)$ -AuP-RTag-1 probes; detection of splice junctions in MDA-MB-231 using $\Delta(9, 10)$ -AuP-RTag-1 and $\Delta(5)$ -AuP-RTag-2 probes; and detection of splice junctions in MDA-MB-231 using $\Delta(9, 10)$ -AuP-RTag-2 and $\Delta(5)$ -AuP-RTag-1 probes. To ensure that the experimental procedure is effective, two positive controls were first performed using fabricated DNA targets $\Delta(9, 10)$ and $\Delta(5)$. In positive control-1, $\Delta(9, 10)$ -AuP-RTag-1 and $\Delta(5)$ -AuP-RTag-2 probes were used; while in positive control-2, $\Delta(9, 10)$ -AuP-RTag-2 and $\Delta(5)$ -AuP-RTag-1 probes were used. As shown in Figure 2, the controls show distinct characteristic peaks attributable to the corresponding Raman tags (1001 cm^{-1} , 1091 cm^{-1} and 1200 cm^{-1} for DNA-AuP-RTag-1; 1295 cm^{-1} and 1349 cm^{-1} for DNA-AuP-RTag-2). These characteristic peaks were also observed for all the detection cases, indicating that the two splice variants are expressed in the chosen breast cancer cell lines. To ensure detection reliability, the same targets were monitored by different RTag for cross-validation. Detection specificity was tested for a control sample using a target solution containing all of the components except the DNA targets. As expected, a very weak nonspecific signal was obtained (Figure 2). It should be noted that higher level of multiplexing (8-plex) has been achieved using DNA-AuP-RTag probes.¹⁴ Therefore, it is possible to monitor more gene segments from the same cell line or different cell lines simultaneously using the proposed strategy to improve detection efficiency.

For quantification, we use the normalizing standard developed in our previous studies^{29,30} based on the plasmon-phonon band to quantify two different DNA targets identified in the duplex detection scheme discussed in Figure 1. Individual sets of capturing strands and DNA-AuP-RTag probes were used to quantify the corresponding DNA targets in the target mixture obtained from different cell lines through DNA/RNA hybridization, S1 nuclease digestion and alkaline hydrolysis. Here we quantify $\Delta(9, 10)$ in the target mixture containing $\Delta(9, 10)$ and $\Delta(5)$ fragments extracted from MCF-7 and MDA-MB-231 cell lines by the SERS sandwich

assay with the respective CS and the probe (RTag-1) for $\Delta(9, 10)$. A control sample without targets was measured on the same slide and the representative Raman spectra are shown in Figure 3. For quantification, baseline correction was first done by subtracting the baseline magnitude (chosen according to the characteristic band of the Raman tag used) from the characteristic band of the tag (1091 cm^{-1} for RTag-1) and the respective plasmon-phonon band (around 220 cm^{-1}) of each spectrum. The corrected characteristic peak (I) was then normalized against the corrected plasmon-phonon band (I_0). The normalized control signal or nonspecific signal was then subtracted from the plasmon-phonon band normalized sample signal and the resulted intensity was used in the calibration equation ($Intensity_{norm} = 6.842 \cdot conc.^{0.2169} + 0.1138$) developed in our previous study³⁰ to evaluate the target concentration. A similar procedure was used to quantify $\Delta(5)$ expressed in both MCF-7 and MDA-MB-231 cells. The estimated DNA targets for splice junctions $\Delta(9, 10)$ and $\Delta(5)$ from both MCF-7 and MDA-MB-231 cells are summarized in Table 2. To validate these results, a conventional RT-PCR method was used to compare the expression levels of the splice junctions $\Delta(9, 10)$ and $\Delta(5)$ from both cell lines qualitatively. Primers for these splice junctions were designed to cross specific exon/exon junctions and a housekeeping gene, β -actin, was used as an internal control for PCR analysis. The quantification results presented in Table 2 reveals that the expression of $\Delta(9, 10)$ was higher in MCF-7 than MDA-MB-231, which agrees well with results from the study of comparing expression profiles of predominant BRCA1 splice variants in tumor and normal cell lines⁵; while the expression of $\Delta(5)$ was lower in MCF-7 than MDA-MB-231. The expression of $\Delta(9, 10)$ was found to be higher than $\Delta(5)$ in MCF-7, and lower than $\Delta(5)$ in MDA-MB-231. The RT-PCR validation shown in Figure 4 confirms the SERS results. It should be noted that customization of the slide could be done to integrate array spots for multiplex detection and quantification with controls as well as any other necessary tests to perform all of the corresponding sandwich assays simultaneously by choosing appropriate sets of capturing and probing strands.

Conclusions

This study demonstrated the use of SERS as an efficient and quantitative analytical tool for breast cancer cell gene expression analysis. The proposed methodology capitalizes on the multiplex sensing capability coupled with highly sensitive and unambiguous detection by SERS using inexpensive nonfluorescent Raman tags to quantify gene fragments without amplification. The strategy also bypasses the need for a highly uniform SERS substrate to achieve reliable quantification. Duplex detection and quantification of BRCA1 expression at the resolution of alternative splicing were successfully achieved. This method could be extended to study any segment of the genome in a multiplex and quantitative manner.

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References

1. Faulds K, Smith WE, Graham D. Analyst 2005;130:1125. [PubMed: 16021211]
2. Bustin SA, Nolan T. Journal of biomolecular techniques 2004;15:155. [PubMed: 15331581]
3. Isola NR, Stokes DL, Vo-Dinh T. Anal. Chem 1998;70:1352. [PubMed: 9553492]
4. Ni J, Lipert RJ, Dawson GB, Porter MD. Anal. Chem 1999;71:4903. [PubMed: 10565281]
5. Orban TI, Olah E. Biochem. Biophys. Res. Commun 2001;280:32. [PubMed: 11162473]
6. Ji H, Davis RW. Nat. Biotechnol 2006;24:1112. [PubMed: 16964224]
7. Mulvaney SP, Musick MD, Keating CD, Natan MJ. Langmuir 2003;19:4784.

8. Qian XM, Nie SM. Chem. Soc. Rev 2008;37:912. [PubMed: 18443676]
9. Graham D, Smith WE, Linacre AMT, Munro CH, Watson ND, White PC. Anal. Chem 1997;69:4703.
10. Faulds K, Smith WE, Graham D. Anal. Chem 2004;76:412. [PubMed: 14719891]
11. Cao YC, Jin R, Mirkin CA. Science 2002;297:1536. [PubMed: 12202825]
12. Wabuyele MB, Vo-Dinh T. Anal. Chem 2005;77:7810. [PubMed: 16316192]
13. Faulds K, Fruk L, Robson DC, Thompson DG, Enright A, Smith WE, Graham D. Faraday Discuss 2006;132:261. [PubMed: 16833121]
14. Sun L, Yu C, Irudayaraj J. Anal. Chem 2007;79:3981. [PubMed: 17465531]
15. Graham D, Mallinder BJ, Whitcombe D, Watson ND, Smith WE. Anal. Chem 2002;74:1069. [PubMed: 11924965]
16. Culha M, Stokes D, Allain LR, Vo-Dinh T. Anal. Chem 2003;75:6196. [PubMed: 14616001]
17. Park T, Lee S, Seong GH, Choo J, Lee EK, Kim YS, Ji WH, Hwang SY, Gweon D-G, Lee S. Lab on a Chip 2005;5:437. [PubMed: 15791342]
18. Faulds K, McKenzie F, Smith WE, Graham D. Angew. Chem., Int. Ed 2007;46:1829.
19. Sun L, Yu C, Irudayaraj J. Anal. Chem 2008;80:3342. [PubMed: 18341356]
20. Bell SEJ, Sirimuthu NMS. Chem. Soc. Rev 2008;37:1012. [PubMed: 18443686]
21. Graham D, Faulds K. Chem. Soc. Rev 2008;37:1042. [PubMed: 18443688]
22. Wang H, Levin CS, Halas NJ. J. Am. Chem. Soc 2005;127:14992. [PubMed: 16248615]
23. Baia M, Baia L, Astilean S. Chem. Phys. Lett 2005;404:3.
24. Sawai Y, Takimoto B, Nabika H, Ajito K, Murakoshi K. J. Am. Chem. Soc 2007;129:1658. [PubMed: 17284005]
25. Lee SJ, Morrill AR, Moskovits M. J. Am. Chem. Soc 2006;128:2200. [PubMed: 16478159]
26. Stokes RJ, Macaskill A, Dougan JA, Hargreaves PG, Stanford HM, Smith WE, Faulds K, Graham D. Chem. Commun 2007:2811.
27. Zhang D, Xie Y, Deb SK, Davisson VJ, Ben-Amotz D. Anal. Chem 2005;77:3563. [PubMed: 15924390]
28. Deb SK, Davis B, Knudsen GM, Gudihal R, Ben-Amotz D, Davisson VJ. J. Am. Chem. Soc 2008;130:9624. [PubMed: 18593116]
29. Shamsaie A, Heim J, Yanik AA, Irudayaraj J. Chem. Phys. Lett 2008;461:131.
30. Sun L, Irudayaraj J. Biophys. J 2009;96:4709. [PubMed: 19486693]
31. Shenk TE, Rhodes C, Rigby PW, Berg P. PNAS 1975;72:989. [PubMed: 165498]
32. Kimura-Suda H, Petrovykh DY, Tarlov MJ, Whitman LJ. J. Am. Chem. Soc 2003;125:9014. [PubMed: 15369348]

Figure 1.
A detection schematic for splice junction profiling in cancer cells.

Figure 2.

Duplex detection of $\Delta(9, 10)$ and $\Delta(5)$ expressed in MCF-7 and MDA-MB-231 cells. Positive control-1: detection of fabricated DNA targets $\Delta(9, 10)$ and $\Delta(5)$ using $\Delta(9, 10)$ -AuP-RTag-1 and $\Delta(5)$ -AuP-RTag-2 probes; Positive control-2: detection of fabricated DNA targets $\Delta(9, 10)$ and $\Delta(5)$ using $\Delta(9, 10)$ -AuP-RTag-2 and $\Delta(5)$ -AuP-RTag-1 probes; Case-1: detection of splice junctions in MCF-7 using $\Delta(9, 10)$ -AuP-RTag-1 and $\Delta(5)$ -AuP-RTag-2 probes; Case-2: detection of splice junctions in MCF-7 using $\Delta(9, 10)$ -AuP-RTag-2 and $\Delta(5)$ -AuP-RTag-1 probes; Case-3: detection of splice junctions in MDA-MB-231 using $\Delta(9, 10)$ -AuP-RTag-1 and $\Delta(5)$ -AuP-RTag-2 probes; Case-4: detection of splice junctions in MDA-MB-231 using $\Delta(9, 10)$ -AuP-RTag-2 and $\Delta(5)$ -AuP-RTag-1 probes.

Figure 3. Representative spectra for quantification of $\Delta(9, 10)$ expressed in MCF-7 and MDA-MB-231 cells.

Figure 4.
RT-PCR analysis of splice junctions $\Delta(9, 10)$ and $\Delta(5)$ from MCF-7 and MDA-MB-231 cells.

Table 1

Oligonucleotide sequences used in experiments

oligonucleotide	sequence (5' – 3')
Δ(9, 10): TS	TCAGAAAATTCACAAGCAGCCAATTCAATGTAGACAGACG
Δ(9, 10): CS	GCTGCTTGTGAATTTTCTGATTTTTTTTTT-C3-SH
Δ(9, 10): PS	SH-C6-TTTTTTTTTTCGTCTGTCTACATTGAATTG
Δ(5): TS	TCGTACTTTCTTGTAGGCTCTTGCAAAATATGTGGTCACA
Δ(5): CS	GAGCCTACAAGAAAGTACGATTTTTTTTTT-C3-SH
Δ(5): PS	SH-C6-TTTTTTTTTTGTGACCACATATTTTGCAA
Δ(9, 10): sense primer	GCAGGAAACCAGTCTCAGTG
Δ(9, 10): anti-sense primer	TTCACAAGCAGCCAATTCAATG (amplicon size: 118 bp)
Δ(5): sense primer	GCAGAAAATCTTAGAGTGTCCTCATCT
Δ(5): anti-sense primer	CGTACTTTCTTGTAGGCTCTTGCAAA (amplicon size: 100 bp)
β-actin: sense primer	CATGTACGTTGCTATCCAGGC
β-actin: anti-sense primer	CTCCTTAATGTCACGCACGAT (amplicon size: 250 bp)

TS: target strand; CS: capturing strand; PS: probing strand.

Table 2Quantification of splice junctions $\Delta(9, 10)$ and $\Delta(5)$ from MCF-7 and MDA-MB-231 cells

	MCF-7	MDA-MB-231
$\Delta(9, 10)$	8.4 pM	2.3 pM
$\Delta(5)$	4.6 pM	32 pM