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Cancer Cell Discrimination Using Host-Guest ‘Doubled’ Arrays

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Abstract

We report a nanosensor that uses cell lysates to rapidly profile the tumorigenicity of cancer cells. This sensing platform uses host-guest interactions between cucurbit[7]uril (CB[7]) and the cationic headgroup of a gold nanoparticle (AuNP) to non-covalently modify the binding of three fluorescent proteins of a multichannel sensor *in situ*. This approach doubles the number of output channels to six, providing single-well identification of cell lysates with 100 % accuracy. Significantly, this classification could be extended beyond the training set, determining the invasiveness of novel cell lines. The unique fingerprint of these cell lysates required minimal sample quantity (200 ng, ~1000 cells), making the methodology compatible with microbiopsy technology.

Graphical Abstract

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Notes

The authors declare no competing financial interest.

Supporting Information. Materials used, experimental methods, statistical analysis, discussion on the affects of CB[7] on sensor components, unknown identification, supporting figures, supporting tables, and data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Keywords

cancer cells; array-based sensing; gold nanoparticle; chemical nose; cell lysates; host-guest chemistry

INTRODUCTION

Rapid methods for geno- and phenotyping cells are crucial for cancer prognosis and the design of therapeutic strategies for precision medicine.¹ Discrimination between healthy and cancerous cells, and then geno/phenotyping to determine whether the cancer is a slow-growing variant or a highly aggressive form are all important for optimal treatment.² The cell proteome provides a significant resource for determining cell tumorigenicity.³ Traditionally, biomarker-based approaches employing proteomics techniques such as electrophoresis and mass spectrometry have been used to detect changes in cell state.⁴ These methods, however, require prior knowledge of the tumor, and are often not sensitive to subtle changes in proteomic signatures. In contrast, array-based ‘chemical nose’ sensing provides an alternative strategy uses selective receptors to generate multiple output channels that are used to create patterns (training sets), analogous to olfaction.⁵ These outputs are then used to build a global diagnostic pattern that can be used to rapidly identify individual small molecule⁶ and biomacromolecular analytes.⁷ More recently, array-based sensing has been used to profile complex biosystems,⁸ including the use of cell lysates to rapidly profile geno/phenotype of cells and tissues for cancer diagnosis.⁹

The traditional application of array-based sensing protocols uses spatially separated sensor units each with their own recognition element to provide the multiple outputs required for pattern generation.¹⁰ Some studies report materials that have multiple optical properties that can be employed to give multiple channels within a single receptor;¹¹ however, even in this format, each optical signal must still be measured separately. In recent research, an alternative strategy employing a single nanoparticle recognition element with three different transducers (red, green, and blue fluorescent proteins (FPs)) was used to generate multi-

channel outputs. This approach greatly simplifies the sensor system physically, facilitating 'one-well' discrimination of complex biosystems, including identification of bacterial biofilms,¹² mammalian cells,^{13 a,b} and determination of drug mechanisms.^{13c} A key challenge with this strategy, however, is generation of sufficient non-interfering channels (e.g. fluorescent emission wavelengths) for effective pattern generation.

Host-guest chemistry is a versatile tool for noncovalent modification of polymers and nanomaterials, altering the structure and concomitantly the behavior of these materials.¹⁴ This approach has been widely used in many applications, including imaging,¹⁵ therapeutic delivery,¹⁶ and sensing.¹⁷ We hypothesized that a host-guest strategy could be used to increase the information content of array-based sensing platforms, facilitating their use in cancer identification and typing. In our approach, we used noncovalent modification of a cationic benzylammonium-functionalized nanoparticle with a complementary cucurbit[7]uril (CB[7]) moiety (Scheme 1).¹⁸ This binding modulates the interaction of the particle with both the fluorescent protein transduction elements and the cell lysate analytes. This change in competitive binding effectively doubles the number of output channels from three to six while maintaining the one-well configuration. This increased information content allowed facile discrimination of cells by their tumorigenicity. Significantly, this classification could be extended beyond the training set, determining the invasiveness of novel cell lines. Full differentiation of cell types was achieved with as little as 200 ng of protein (~1000 cells), demonstrating the potential of this method for microbiopsy-based cancer diagnostics.

RESULTS AND DISCUSSION

The sensor consists of two supramolecularly-related recognition receptors: BenzNP and BenzNP-CB[7]. BenzNP can bind to the CB[7] host molecule to rapidly form host-guest complexes.¹⁹ The binding events of BenzNP and BenzNP-CB[7] with the lysates are transduced by three fluorescent proteins: blue (EBFP2) (B), green (EGFP) (G), and red (tdTomato) (R).^{13c} In the presence of BenzNP, the fluorescence intensity of FPs is quenched. Different amounts of FPs are then released after the addition of cell lysates due to their competitive binding for the particles, generating channels 1–3. Host-guest interactions between CB[7] and BenzNP²⁰ create the second recognition receptor.²¹ The addition of CB[7] triggered a drastic change in fluorescence intensity of the three FPs, indicating a stronger interaction of BenzNP-CB[7] complexes to FPs than the BenzNP,²² generating channel 4–6. CB[7] is a good synthetic receptor for peptides and proteins, potentially contributing to the enhanced quenching ability of BenzNP-CB[7] toward FPs in the presence of lysates.²³ To ensure that the fluorescence changes are induced by the interaction of BenzNP-CB[7] with cell lysates and not from any changes of FPs themselves, we tested the effect of CB[7] and lysates on FPs alone and found that they do not affect fluorescence intensity of FPs (Figure S1, S2).

The first step in our sensing was to determine the appropriate conditions for converting Benz-NP to BenzNP-CB[7]. The appropriate stoichiometry of CB[7] and BenzNP was determined by isothermal titration calorimetry (ITC) and the 100/1 ratio of CB[7]/BenzNP was used throughout our sensing experiments (Figure S3, S4).

As an initial test of our method, five human cancer cell lines with different tissue/organ origins were used: MCF-7 (breast), SKOV3 (ovarian), Raji (blood), NCI-H1299 (lung), HEK239T (kidney) (Table 1). We used fluorescence spectroscopy to generate “fingerprints” for the cell lysates obtained from the cultured cell lines. In practice, cell lysates were added to the BenzNP-FPs complex, readings taken, then CB[7] was added to generate BenzNP-CB[7] in situ, with subsequent readout. These fluorescence outputs were analyzed by linear discriminant analysis (LDA). Cluster separation on an LDA plot was generated based on their standardized Mahalanobis distance: the greater the distance the lower the probability of misclassification. When only three channels are used, there is substantial overlap among different cell lines, especially NCI-H1299, SKOV3, MCF7, and Raji for the BenzNP-FPs channels (Figure 1a), and overlaps between NCI-H1299 with HEK-239T, and SKOV3 with MCF-7 for the BenzNP-CB[7] channels (Figure 1b). However, when all 6 channels are combined, all five cell lines are well separated (Figure 1c).

One critical step in chemical-nose sensing is to challenge its reproducibility. For this purpose, we revalidated our sensor by using an unknown set of all five cell lines (5 cell lines $\times$ 8 replicates = 40 unknown cases). We were able to predict the identities of the 38 out of 40 unknown cases with 95 % correct unknown identification (% CUI) in a single-well configuration using 6-channel system. When there are only three channels, the % CUI drops drastically (Figure 1d).

An important challenge in cancer therapy is determining whether tissue/cells are benign or cancerous. If they are cancerous, then whether or not it has the ability to metastasize to other organs. We chose three different human breast cell lines to test our host-guest doubled array sensor: MCF10A (normal), MCF-7 (cancerous), MDA-MB-231 (metastatic) (Figure 2a). The three cell lines also show differential fluorescence patterns that are clustered separately by LDA when 6 channels are used (Figure 2b). Similar trend is observed in the % CUI of these three human breast cell lines where the six channel system gives much better unknown identification of 96 % (23 out of 24 samples) compared to the three channels (Figure 2c).

To further validate our sensor with a more stringent test, we employed isogenic cell lines derived from BALB/c mice. These cells can provide a testbed for our sensor by avoiding the issue of individual-to-individual geo/phenotypic variation since they share the same genetic background but have different histological stages, as characterized *in vivo*. TM40A has undetectable tumorigenicity (0 %), TM9 generated tumors in 38 % of the cases, while MC7-L1 quickly developed tumors and became highly metastatic in 100% of tested mice (100% tumor) (Table 2). These challenging isogenic cell lines are also differentiated using our six channel system (Figure 3a). However, when only three channels are used, there is always some level of overlap between the non-tumorigenic (TM40A) and the low-tumorigenic group (TM9), while the high tumorigenic cluster is highly separated from the others (Figure S3). This indicates biological similarity between the non-tumorigenic and the low-tumorigenic samples. This same trend is also observed in the fluorescence heat map, analyzed by hierarchical clustering analysis (HCA) using six channels, where TM40A while is separated from TM9, they still share the same sub branch with each other and are much more different than the high-tumorigenic cell line MC7-L1 (Figure 3b). These results of 10 cell lines confirm the benefit of increasing the number of channels in chemical-nose sensing

system, not just for the classification of each group but most importantly, the accuracy in unknown identification.

To further validate the versatility of our 6-channel system, we cultured two additional cell lines that have similar histological outcomes with the cell lines in our reference set but different identities. These cell lines are FSK7 (low-tumorigenic) and MC4-L2 (high-tumorigenic).²⁴ The fingerprints of these two cell lines were compared to the reference set to predict their histological outcomes. LDA plots show the overlap of each unknown cell line with their corresponding tumorigenicity with 100 % accuracy (Figure 4, Table S6). This result strengthens the sensor reliability in identifying clinically relevant features of cells. Notably, translating results from one cell line to use as reference for other novel cell lines has not been achieved previously with chemical-nose sensors.

CONCLUSIONS

In summary, we have used host-guest chemistry to double the information content of an array-based sensor for cancer diagnostics. The sensor was able to readily discriminate phenotypic changes among cells based on their complex proteomic signatures associated with different histological outcomes. The unique fingerprint of complex cell lysates can be obtained in a single well with minimal sample quantity (200 ng of total proteins), minimizing biopsy size, reducing the invasiveness of the methodology. Significantly, the classification of this host-guest-based sensor could be extended beyond the training set, determining the invasiveness of unknown cell lines that have similar histological outcomes. In a broader context, this host-guest-based chemical nose sensor presents a general means of increasing dimensionality in array-based sensors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. (a) Van de Stolpe A, Den Toonder MJM. *Cancers*. 2014; 6:1195–1207. [PubMed: 24879438] (b) Reil A, Wesche J, Greinacher A, Bux J. *Transfusion*. 2011; 51:18–24. [PubMed: 20561293]
2. (a) Peng G, Tisch U, Adams O, Hakim M, Shehada N, Broza YY, Billan S, Abdah-Bortnyak R, Kuten A, Haick H. *Nat Nanotechnol*. 2009; 4:669–673. [PubMed: 19809459] (b) Diamandis EP. *Mol Cell Proteomics*. 2004; 3:367–378. [PubMed: 14990683]
3. (a) Everley PA, Krijgsveld J, Zetter BR, Gygi SP. *Mol Cell Proteomics*. 2004; 3:729–735. [PubMed: 15102926] (b) Gharbi S, Gaffney P, Yang A, Zvelebil MJ, Cramer R, Waterfield MD, Timms JF. *Mol Cell Proteomics*. 2002; 1:91–98. [PubMed: 12096126]
4. (a) O'Dwyer D, Ralton LD, O'Shea A, Murray GI. *Plos One*. 2011; 6.(b) Abbani, MA., Mallick, P., Vogelsang, MS. *Mass Spectrometry Based Proteomics in Cancer Research*. Springer; New York: 2010. (c) Hanash S, Taguchi A. *Nature Reviews Cancer*. 2010; 10:652–660. [PubMed: 20733593]

5. (a) Miranda OR, Creran B, Rotello VM. *Curr Opin Chem Biol.* 2010; 14:728–736. [PubMed: 20801707] (b) Wright AT, Anslyn EV. *Chem Soc Rev.* 2006; 35:14–28. [PubMed: 16365639] (c) Tao Y, Ran X, Ren J, Qu X. *Small.* 2014; 10:3667–3671. [PubMed: 24840238]
6. (a) Peng G, Tisch U, Adams O, Hakim M, Shehada N, Broza YY, Billan S, Abdah-Bortnyak R, Kuten A, Haick H. *Nat Nanotechnol.* 2009; 4:669–673. [PubMed: 19809459] (b) Palacios MA, Nishiyabu R, Marquez M, Anzenbacher P. *J Am Chem Soc.* 2007; 129:7538–7544. [PubMed: 17530846] (c) Kumar V, Anslyn EV. *J Am Chem Soc.* 2013; 135:6338–6344. [PubMed: 23544452]
7. (a) Zhou H, Baldini L, Hong J, Wilson AJ, Hamilton AD. *J Am Chem Soc.* 2006; 128:2421–2425. [PubMed: 16478197] (b) De M, Rana S, Akpinar H, Miranda OR, Arvizo RR, Bunz UHF, Rotello VM. *Nat Chem.* 2009; 1:461–465. [PubMed: 20161380]
8. (a) Bajaj A, Miranda OR, Kim IB, Phillips RL, Jerry DJ, Bunz UHF, Rotello VM. *Proc Natl Acad Sci.* 2009; 106:10912–10916. [PubMed: 19549846] (b) El-Boubbou K, Zhu DC, Vasileiou C, Borhan B, Prosperi D, Li W, Huang X. *J Am Chem Soc.* 2010; 132:4490–4499. [PubMed: 20201530] (c) Phillips RL, Miranda OR, You CC, Rotello VM, Bunz UHF. *Angew Chem Int Ed.* 2008; 47:2590–2594.
9. Rana S, Singla AK, Bajaj A, Elci SG, Miranda OR, Mout R, Yan B, Jirik FR, Rotello VM. *ACS Nano.* 2012; 6:8233–8240. [PubMed: 22920837]
10. (a) You CC, Miranda OR, Gider B, Ghosh PS, Kim IK-B, Erdogan B, Krovi SA, Bunz UHF, Rotello VM. *Nat Nanotechnol.* 2007; 2:318–323. [PubMed: 18654291] (b) Chou SS, De M, Luo J, Rotello VM, Huang J, Druvid VP. *J Am Chem Soc.* 2012; 134:16725–16733. [PubMed: 22962967] (c) Pei H, Li HJ, Lv M, Wang J, Gao J, Lu J, Li Y, Huang Q, Hu J, Fan C. *J Am Chem Soc.* 2012; 134:13843–13849. [PubMed: 22849568] (d) Yuan Z, Du Y, Tseng YT, Peng M, Cai N, He Y, Chang HT, Yeung ES. *Anal Chem.* 2015; 87:4253–4259. [PubMed: 25824850] (e) Kong H, Liu D, Zhang SC, Zhang XR. *Anal Chem.* 2011; 83:1867–1870. [PubMed: 21323355]
11. (a) Jiménez D, Martínez-Máñez R, Sancenón F, Ros-Lis JV, Soto J, Benito Á, García-Breijo E. *Eur J Inorg Chem.* 2005; 2005:2393–2403. (b) Ábalos T, Jiménez D, Martínez-Máñez R, Ros-Lis JV, Royo S, Sancenón F, Soto J, Costero AM, Gil S, Parra M. *Tetrahedron Lett.* 2009; 50:3885–3888. (c) Jiménez D, Martínez-Máñez R, Sancenón F, Soto J. *Tetrahedron Lett.* 2004; 45:1257–1259. (d) Wu P, Miao L-N, Wang H-F, Shao X-G, Yan X-P. *Angew Chem Int Ed.* 2011; 50:8118–8121. (e) Schmittel M, Lin H-W. *Angew Chem Int Ed.* 2007; 46:893–896.
12. Li X, Kong H, Mout R, Saha K, Moyano DF, Robinson SM, Rana S, Zhang X, Riley MA, Rotello VM. *ACS Nano.* 2014; 8:12014–12019. [PubMed: 25454256]
13. (a) Rana S, Le NDB, Mout R, Duncan B, Elci SG, Saha K, Rotello VM. *ACS Cent Sci.* 2015; 1:191–197. [PubMed: 26405691] (b) Xu Q, Zhang Y, Tang B, Zhang CY. *Anal Chem.* 2016; 88:2051–2058. [PubMed: 26759896] (c) Rana S, Le NDB, Mout R, Saha K, Tonga GY, Bain RES, Miranda OR, Rotello CM, Rotello VM. *Nat Nanotechnol.* 2015; 10:65–69. [PubMed: 25502312]
14. (a) Grana-Suarez L, Verboom W, Huskens J. *Chem Commun.* 2014; 50:7280–7282. (b) Yang SK, Ambade AV, Weck M. *J Am Chem Soc.* 2010; 132:1637–1645. [PubMed: 20078047] (c) Chandler D. *Nature.* 2005; 437:640–647. [PubMed: 16193038] (d) Wenz G, Han BH, Müller A. *Chem Rev.* 2006; 106:782–817. [PubMed: 16522009]
15. Xu XD, Zhao L, Qu Q, Wang JG, Shi H, Zhao Y. *ACS Appl Mater Interfaces.* 2015; 7:17371–17380. [PubMed: 26186168]
16. Hu QD, Tang GP, Chu PK. *Acc Chem Res.* 2014; 47:2017–2025. [PubMed: 24873201]
17. Esser B, Swager TM. *Angew Chem, Int Ed.* 2010; 49:8872–8875.
18. (a) Lee JW, Samal S, Selvapalam N, Kim HJ, Kim K. *Acc Chem Res.* 2003; 36:621–630. [PubMed: 12924959] (b) Masson E, Ling X, Joseph R, Kyeremeh-Mensah L, Lu X. *RSC Adv.* 2012; 2:1213–1247.
19. Kim C, Tonga GY, Yan B, Kim CS, Kim ST, Park MH, Zhu Z, Duncan B, Creran B, Rotello VM. *Org Biomol Chem.* 2015; 13:2474–2479. [PubMed: 25569869]
20. Freeman WA, Mock WL, Shih NY. *J Am Chem Soc.* 1981; 103:7367–7368.
21. (a) Cao L, Isaacs L. *Supramol Chem.* 2014; 26:251–258. (b) Zhao J, Zhang YM, Sun HL, Chang XY, Liu Y. *Chem Eur J.* 2014; 20:15108–15115. [PubMed: 25284456]
22. (a) Yeh YC, Rana S, Mout R, Yan B, Alfonso FS, Rotello VM. *Chem Commun.* 2014; 50:5565–5568. (b) Bhasikuttan AC, Mohanty J, Nau WM, Pal H. *Angew Chem.* 2007; 119:4198–4200. (c)

- Chinai JM, Taylor AB, Ryno LM, Hargreaves ND, Morris CA, Hart PJ, Urbach AR. *J Am Chem Soc.* 2011; 133:8810–8813. [PubMed: 21473587]
23. Logsdon LA, Schardon CL, Ramalingam V, Kwee SK, Urbach AR. *J Am Chem Soc.* 2011; 133:17087–17092. [PubMed: 21967539]
24. (a) Kittrell FS, Oborn CJ, Medina D. *Cancer Res.* 1992; 52:1924–1932. [PubMed: 1372532] (b) Medina D. *J Cell Biochem.* 1993:155–155.

Figure 1.

Five human cancerous cell lines were clustered using Linear Discriminant Analysis (LDA) with the fluorescence responses from a) only three channels: BenzNP-FPs and b) BenzNP-CB[7], c) all six channels. d) Correct unknown identification percentage of three sensing systems. Unknown population is 40 samples for five cell lines. (Table S1–S4).

Figure 2.

Three human breast cell lines with a) different cell status were clustered using Linear Discriminant Analysis (LDA) with the fluorescence responses from b) six channels. c) Correct unknown identification percentage of three sensing systems. Unknown population is 24 samples for three cell lines. (Table S5–S8).

Figure 3.

Three isogenic breast cell lines derived from BALB/c mice were clustered using Linear Discriminant Analysis (LDA) with the fluorescence responses from a) six channels. b) Heat map of the fluorescence response patterns for the reference set using six channels. Hierarchical clustering was performed on the normalized average of the fluorescence responses, where I_0 is the initial fluorescence intensity of the sensor and I is the final fluorescence intensity of the sensor after lysate incubation. c) Correct unknown identification percentage of three sensing systems. Unknown population is 24 samples for three cell lines. (Table S9–S12).

Figure 4.

a) Unknown cell lines were clustered with the established reference cell lines via LDA by using the fluorescence responses from all six channels. b) The correct unknown identification percentage of FSK7 and MC4-L2 are both 100 % (Table S13).

Scheme 1.

Six channel-output in a single well.. The fluorescence of FPs is quenched when the BenzNP-FP complexes are formed. Upon addition of cell lysates, three emission channels are obtained from the released FPs. In the same well, CB[7] is added to obtain three additional channels from the three FPs as a result of changed interactions between the analyte and newly formed complex, BenzNP-CB[7].

Table 1

Features of five cancerous human cell lines with different tissue origins

#	Cell line	Tissue origin	Cell status
1	MCF-7	Breast	Cancerous
2	SKOV3	Ovarian	Cancerous
3	Raji	Blood	Cancerous
4	NCI-H1299	Lung	Cancerous
5	HEK239T	kidney	Cancerous

Table 2

Features of five breast cell lines

	#	Cell line	Tissue origin	Cell status
Reference cell lines	1	TM40A	Breast	Normal
	2	TM9	Breast	Low-tumorigenic
	3	MC7-L1	Breast	High-tumorigenic
Cell lines outside of reference	4	FSK7	Breast	Low-tumorigenic
	5	MC4-L2	Breast	High-tumorigenic