Electronic Supplementary Material (ESI) for ChemComm.


# Supplementary Information

## Element Probe Based CRISPR/Cas14 Bioassay for Non-Nucleic-Acid Targets

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## Experimental section

### Materials and Instrumentations

TracrRNA was purchased from Tolo Biotech. (Shanghai, China). CrRNA was synthesized and purified by Takara Bio (Beijing, China). The rest oligonucleotides were synthesized and HPLC purified by Shanghai Sangon Biotechnology Co. Ltd. All oligonucleotides were listed in Table S1. 1,4,7,10-tetraazacyclododecane-1,4,7-tris-aceticacid-10-maleimidoethylacetamide (MMA-DOTA) was purchased from MacroCyclics (Dallas, TX), USA (Figure S2). $TbCl_3 \cdot 6H_2O$, $CH_3COONH_4$ were purchased from Sigma-Aldrich (Shanghai, China). Cas14 and the corresponding Cas14 reaction buffer were purchased from Tolo Biotech (Shanghai, China). HEPES buffer (2M, nuclease free), strepavidin-coated magnetic beads (SA-MBs), 4S Red Plus nucleic acid dye, electrophoresis loading buffer and TBE buffer were all purchased from Shanghai Sangon Biotechnology Co. Ltd. DEPC treated nuclease free water was used in all the related buffer for CRISPR/Cas14 cleavage reactions. In addition, deionized water (18.2MΩ $cm^{-1}$) was produced by a Mili-Q ultrapure system and used in 2×B&W buffer (10mM Tris-HCl, 1mM EDTA, 2M NaCl, pH 7.5).

Fluorescence characterization was performed using Cytation™ 5 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc.). And $^{159}Tb$ isotope detection was carried out with NexION 350 quadrupole ICPMS (PerkinElmer, Inc). The optimal work conditions of ICPMS were tuned and listed in Table S2. Thermo-LTQ-MS ion trap mass spectrometer and AXIMA Performance matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) were employed for the characterization of element tagging. The matrix of MALDI-TOF characterization was 3-hydroxy pyridinic acid (3-HPA). High performance liquid chromatography (HPLC) was performed using P230II (Dalian Elite Analytical Instruments Co., Ltd.) and AdvanceBio Oligonucleotide column (HPH-C18, 4.6mm ×150mm, Agilent Technologies Co., Ltd.). Fluochem M (Cell Biosciences, Santa Clara, CA) was used to imaging the electrophoresis gel. Scanning electron microscopy (SEM, Hitachi, S3400) was utilized to collect X-ray

energy spectrum (EDS) information for the characterization of SA- MBs surface elements distribution before and after the reaction.

**Synthesis and purification of Tb-Rep**

As shown in Figure. S2, $Tb^{3+}$ ion was first chelated with equimolar MMA-DOTA in ammonium acetate buffer (500mM, pH 5.5) and incubated at 37 ℃ for 30 min. The pre-reaction MMA-DOTA and post-reaction DOTA/Tb were characterized by ESI-MS, respectively (Figure S5). Next, 25× excessive presynthesized MMA-DOTA-Tb was mixed with the thiol group modified single strand DNA (ssDNA) in ammonium acetate buffer (500mM, pH 5.5) and oscillated for 90 minutes at 37 ℃ (Figure S4). The final ssDNA-DOTA-Tb (Tb-Rep) was desalted and purified by HPLC. The chromatographic gradient elution was performed on a binary pump, and the eluted components were collected and then characterized by MALDI-TOF-MS. Mobile phase A was DIW, while mobile phase B was chromatographically pure acetonitrile. Elution condition was that the mobile phase ratio decreased linearly from 90% A to 86% A in 10 min and the flow rate of mobile phase was 0.5mL/min at 260nm wavelength UV monitoring. The purified Tb-Rep was then redissolved in HEPES buffer (pH 7.4, 40mM HEPES, 100mM NaCl, 20mM $MgCl_2$). MALDI-TOF-MS results also confirm the successful synthesis of Tb-Rep of various lengths (Figure S6 and Table S3).

**Element probe based CRISPR/Cas14 sensing platform for Ampicillin detection.**

First, 2μM Aptamer-1 and Aptamer-2 were hybridized with 1μM Activator in HEPES buffer to form the "locked-activated" system (Figure 1). Two times excessive concentration ensures the Aptamer-1 and Aptamer-2 could "lock" the free Activator as many as possible to improve the signal-to-noise ratio. The hybridization was performed in PCR at 95℃ for 5 min and then slowly cooled down to room temperature.

After "locked-activated" system construction, the next is the identification of AMP. Locked Activator was mixed with target AMP in diluted Cas14 buffer followed by introducing a final concentration of 0.25μM

of Cas14, 1μM of tracrRNA-crRNA and 0.25μM of Tb-Rep. Whole identification system was incubated at 25℃ for 90 min, then heated to 80℃ for 5 min to terminate the reaction.

The last is the detection process. The identification system was mixed with equal volume of 2×B&W buffer, and introduced 5μl 10 mg $mL^{-1}$ SA-MBs. Emerging Tb peak after the specific link between biotin and streptavidin demonstrated the successful capture of remaining Tb-Rep in EDS results (FigureS7). SA-MBs could complete the capture within 30 min incubating at 25℃. After magnetic separation, the supernate was acidified to 1% with $HNO_3$ and loaded for ICP-MS detection.

**Polyacrylamide gel electrophoresis and imaging**

All gel electrophoresis experiments (Native-PAGE) utilized 20% polyacrylamide gel. The corresponding 30mL system consisted of 3mL of 10×TBE buffer (890mM Tris, 890mM boric acid, 20mM EDTA, pH 8.3), 20mL of 30% Acr-Bis (30:1) gel and 7mL DEPC treated $H_2O$. Then 300μL 10% ammonium persulfate and 30μL N,N,N',N'-tetramethylenediamine (TEMED) were rapidly mixed. And the polymerization was initiated at 37℃ for 1 hour. Before electrophoresis, each sample was mixed with loading buffer as 6:1. The electrophoresis was performed at a constant voltage of 200 V for about 2 hours. After 1 hour of dyeing with 4S Red Plus, imaging was performed by Fluorchem M imager.

**Table S1** Oligonucleotides sequence (5`-3`)

| | |
|---|---|
| Activator | CAACCGCCCGCAGTTACATTAGTTGACCGCTATACAA |
| Aptamer-1 | GCGGGCGGTTGTATAGCGGTCAACT |
| Aptamer-2 | AATGTAACTGCGGGCGGTTGTATAGCGG |
| tracrRNA | CUUCACUGAUAAAGUGGAGAACCGCUUCACCAAAAGCUGU CCCUUAGGGGAUUAGAACUUGAGUGAAGGUGGGCUGCUU GCAUCAGCCUAAUGUCGAGAAGUGCUUUCUUCGGAAAGU AACCCUCGAAACAAAUUCAUUUGGAAUGCAAC |
| crRNA | GAAUGAAGGAAUGCAACUCAACUAAUGUAACU |
| T5-FAM-Rep | `6 FAM-TTTTT-BHQ1 |
| T6-FAM-Rep | `6 FAM-TTTTTT-BHQ1 |
| T8-FAM-Rep | `6 FAM-TTTTTTTT-BHQ1 |
| T10-FAM-Rep | `6 FAM-TTTTTTTTTT-BHQ1 |
| T12-FAM-Rep | `6 FAM- TTTTTTTTTTTT -BHQ1 |
| T15-FAM-Rep | `6 FAM- TTTTTTTTTTTTTTT -BHQ1 |
| 5-Tb-Rep | SH-TTTTT-biotin |
| 6-Tb-Rep | SH-TTTTTT-biotin |
| 8-Tb-Rep | SH-TTTTTTTT-biotin |
| 10-Tb-Rep | SH-TTTTTTTTTT-biotin |
| 12-Tb-Rep | SH-TTTTTTTTTTTT-biotin |
| 15-Tb-Rep | SH-TTTTTTTTTTTTTTT-biotin |

Underline: AMP nucleic acid aptamer sequence

**Table S2** Operating Conditions of NexION 350 ICP-MS

| Conditions | Settings |
|---|---|
| Radiofrequency power | 1300 W |
| Plasma argon gas flow | 18.00 L $min^{-1}$ |
| Auxiliary argon gas flow | 1.20 L $min^{-1}$ |
| Nebulizer argon gas flow | 0.78 L $min^{-1}$ |
| Resolution | 2060 |
| Dwell time | 50 ms |
| Dead time | 35 ns |
| Sweeps per reading | 120 |
| Isotope monitored | $^{159}Tb$ |

**Table S3** Comparison of theoretical and detected MW of several n-Tb-Rep probes.

| Probes | Theoretical MW | Detected MW |
|---|---|---|
| 5-Tb-Rep | 2716.7 | 2714.05 |
| 6-Tb-Rep | 3020.9 | 3104.52 |
| 8-Tb-Rep | 3629.6 | 3697.04 |
| 10-Tb-Rep | 4237.8 | 4290.08 |
| 12-Tb-Rep | 4846.2 | 5004.97 |
| 15-Tb-Rep | 5758.8 | 5866.58 |

**Table S4** Comparison of aptamer-based assays of ampicillin detection.

| Analytical strategy | Aptamer sequence(5`-3`) | Real sample | LOD (nM) | Ref. |
|---|---|---|---|---|
| Fluorescence | GCGGGCGGTTGTATAGCGG | Milk | 5.7 | 1 |
| Colorimetry | GCGGGCGGTTGTATAGCGG | Milk | 28.6 | 1 |
| Fluorescence | GCGGGCGGTTGTATAGCGGTTTTTTT | River | 0.2 | 2 |
| EIS | GCGGGCGGTTGTATAGCGG | Milk | 0.1 | 3 |
| AED | TTAGTTGGGGTTCAGTTG | BSA | 1000 | 4 |
| AED | TTAGTTGGGGTTCAGTTGG | Urine, water | 30 | 5 |
| ICP-MS | Aptamer-1: GCGGGCGGTTGTATAGCGGTCAACT Aptamer-2: AATGTAACTGCGGGCGGTTGTATAGCGG | River | 2.06 | This work |

* EIS: Electrochemical Impedance Spectroscopy; AED: Amperometric electrochemical detection

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**Table S5** P values and the heatmap of selectivity experiment.

| □ | AMP | SMZ | Kan | GM | Tet | SM | Gly | Tyr | Cys | BLK |
|---|---|---|---|---|---|---|---|---|---|---|
| AMP | □ | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| SMZ | <0.0001 | □ | 0.073 | 0.068 | 0.606 | 0.787 | 0.378 | 0.615 | 0.165 | 0.927 |
| Kan | <0.0001 | 0.073 | □ | 0.277 | 0.24 | 0.835 | 0.809 | 0.357 | 0.383 | 0.05 |
| GM | <0.0001 | 0.068 | 0.277 | □ | 0.65 | 0.294 | 0.665 | 0.211 | 0.856 | 0.061 |
| Tet | <0.0001 | 0.606 | 0.24 | 0.65 | □ | 0.616 | 0.319 | 0.863 | 0.166 | 0.618 |
| SM | <0.0001 | 0.787 | 0.835 | 0.294 | 0.616 | □ | 0.753 | 0.583 | 0.483 | 0.365 |
| Gly | <0.0001 | 0.378 | 0.809 | 0.665 | 0.319 | 0.753 | □ | 0.365 | 0.619 | 0.223 |
| Tyr | <0.0001 | 0.615 | 0.357 | 0.211 | 0.863 | 0.583 | 0.365 | □ | 0.223 | 0.158 |
| Cys | <0.0001 | 0.165 | 0.383 | 0.856 | 0.166 | 0.483 | 0.619 | 0.223 | □ | 0.014 |
| BLK | <0.0001 | 0.927 | 0.05 | 0.061 | 0.618 | 0.365 | 0.223 | 0.158 | 0.014 | □ |

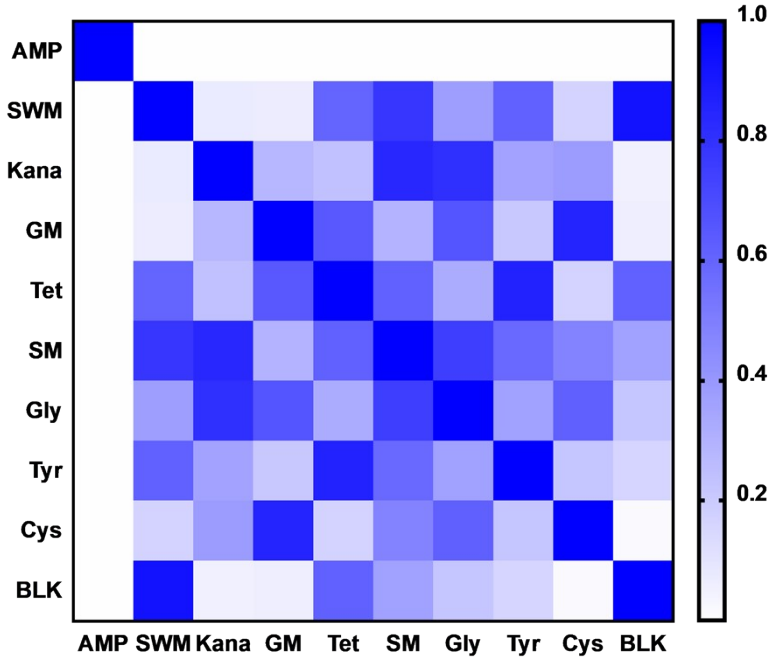


**Table S6** Recovery experiment of AMP spike in aquatic samples.

| AMP added （nM） | Jialing river samples | |
|---|---|---|
| | Detection result (nM) | Recovery (%) |
| 10 | 9.05 | 91 |
| 20 | 18.38 | 92 |
| 30 | 27.24 | 91 |
| 40 | 42.30 | 106 |
| 50 | 55.98 | 112 |

**Figure S1** The secondary structures of (a) Activator, (b) activator-crRNA and (c) "lock-activated" system, simulated by Nupack (www.nupack.org).

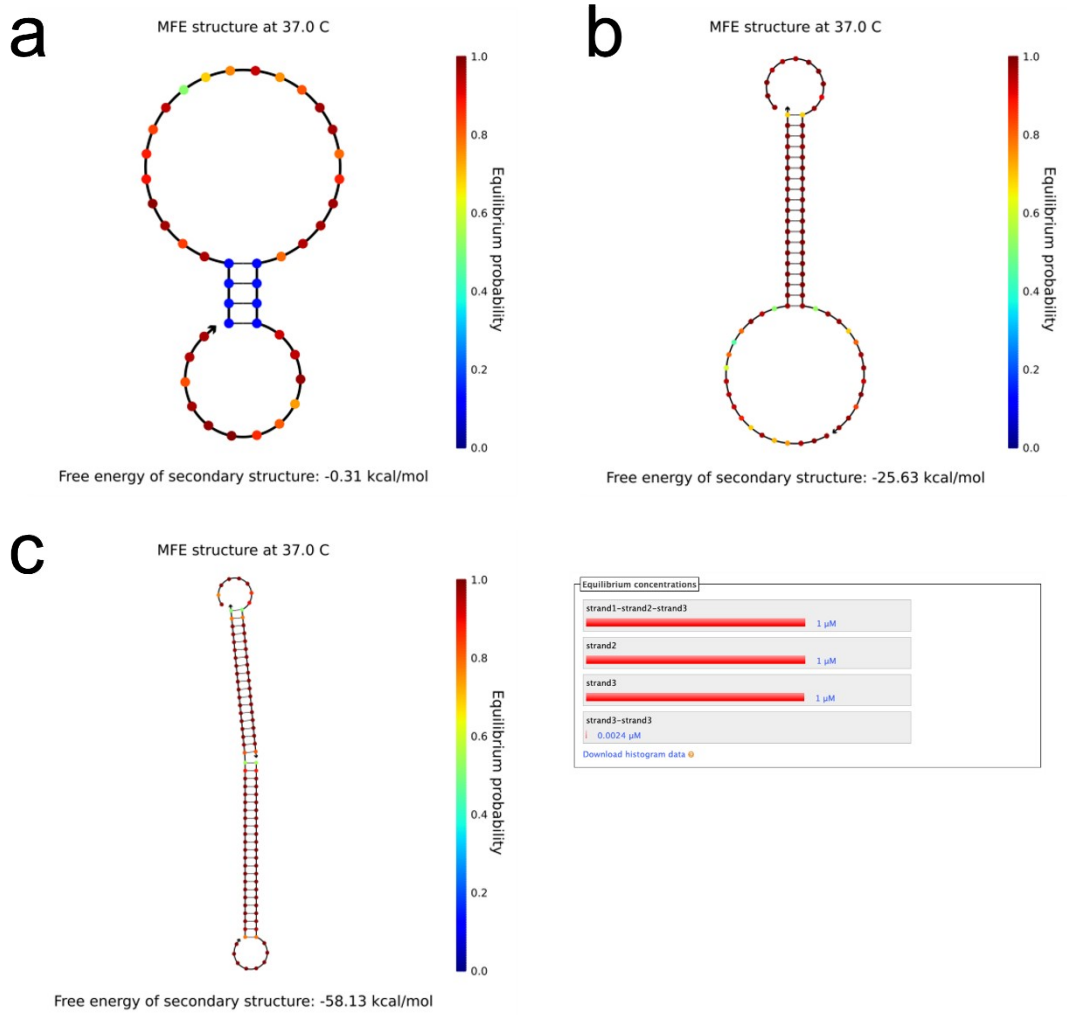


**Figure S2** Chemical structure of maleimido-monomide-DOTA (MMA-DOTA)

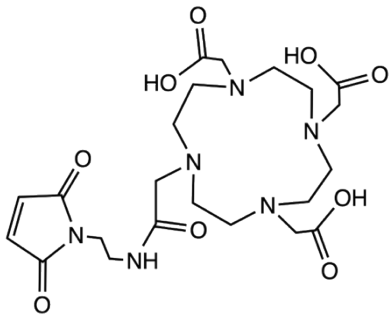


**Figure S3** The mechanism of MMA-DOTA chelating $Tb^{3+}$ ion.

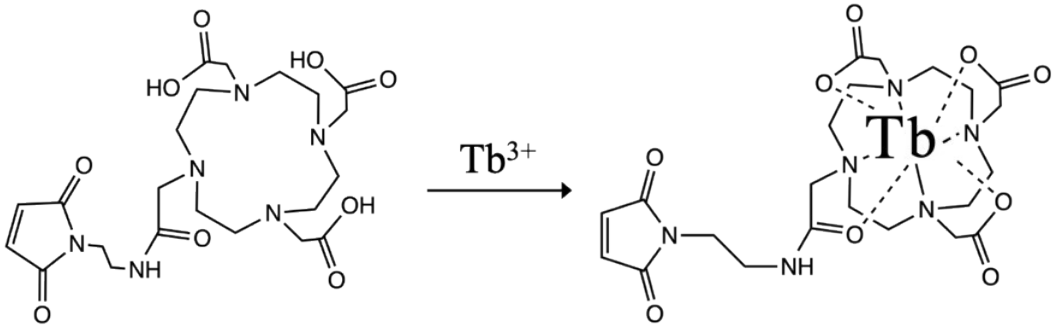



**Figure S4** The mechanism of MMA-DOTA-Tb coupled with SH-ssDNA.

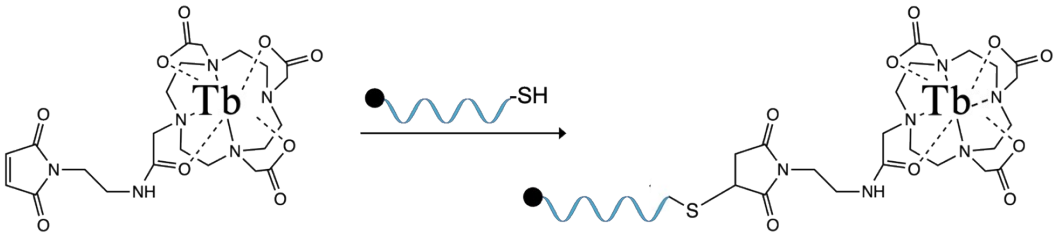


**Figure S5** ESI-MS spectra of MMA-DOTA chelated with $Tb^{3+}$. (a) Before and (b) after reaction.

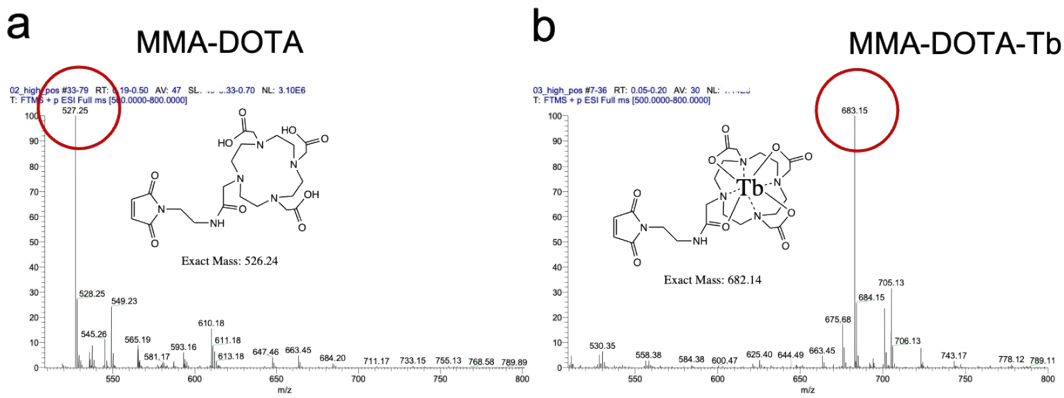


**Figure S6** MALDI-TOF-MS spectra of several n-Tb-Rep probes.

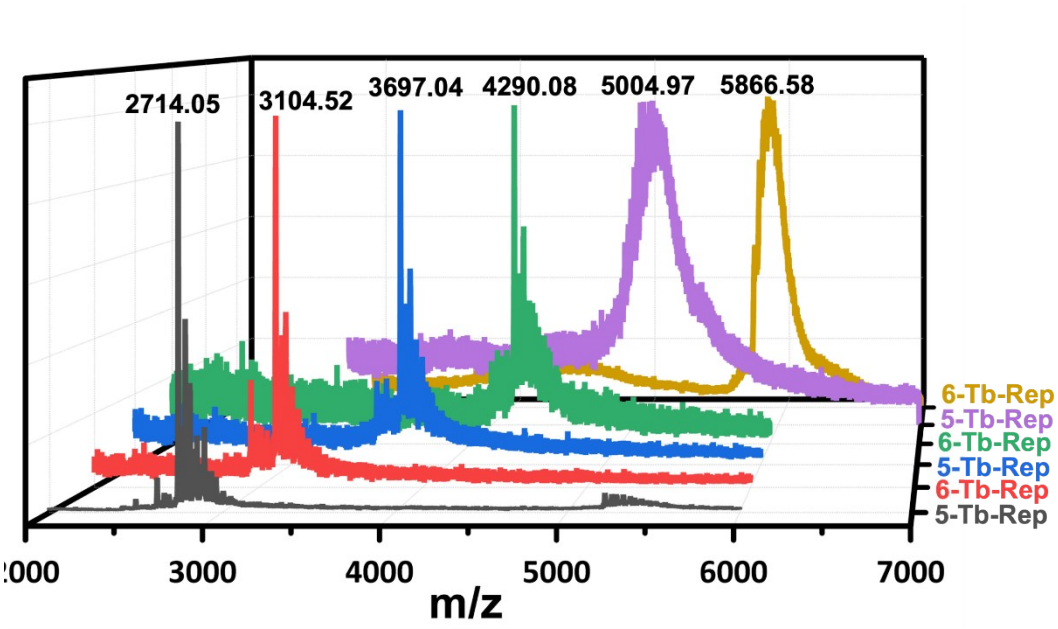
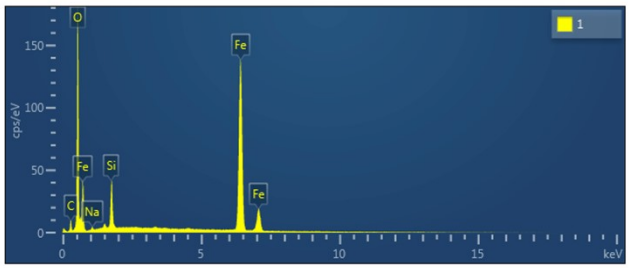


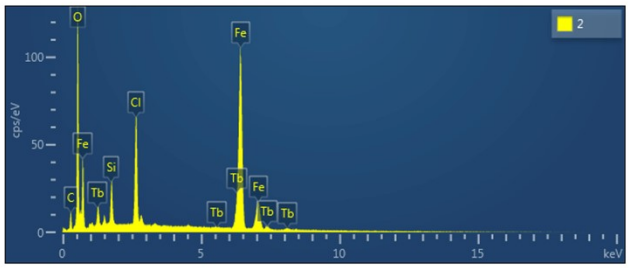
**Figure S7** EDS mapping of Original Sa-Mbs. (b) EDS mapping of Sa-Mbs captured Tb-rep.

a




| Element | Wt% | Atom% |
|---|---|---|
| C | 5.59 | 13.01 |
| O | 29.53 | 51.65 |
| Na | 0.65 | 0.79 |
| Si | 4.75 | 4.74 |
| Fe | 59.48 | 29.80 |
| Total | 100.00 | 100.00 |

b




| Element | Wt% | Atom% |
|---|---|---|
| C | 8.13 | 19.35 |
| O | 26.78 | 47.83 |
| Si | 3.01 | 3.06 |
| Cl | 8.00 | 6.45 |
| Fe | 40.96 | 20.96 |
| Tb | 13.12 | 2.36 |
| Total | 100.00 | 100.00 |