

Supporting Information

Molecularly Imprinted Polymers: Antibody Mimics for Bioimaging and Therapy

Karsten Haupt*, Paulina X. Medina Rangel, Bernadette Tse Sum Bui*

Université de Technologie de Compiègne, CNRS Enzyme and Cell Engineering Laboratory, Rue Roger Couffolenc, CS 60319, 60203 Compiègne Cedex, France

Corresponding authors: jeanne.tse-sum-bui@utc.fr (B. Tse Sum Bui); karsten.haupt@utc.fr (K. Haupt)

Table S1A. MIP-NPs for targeted imaging

Ref.	Biomarker	Molecular template	Imprinting technique	Size DLS (nm)/ ζ potential (mV)	K_d (solvent)	Fluoroprobe	Cell line (<i>in vitro</i>)	Xenograft/ No. mice	Cell viability assay (MIP _{max} μ g/mL)	Flow cytometry
1	Hyaluronic acid	Glucuronic acid	Precipitation polymerization	400/ +48	17.3 μ M (CH ₃ OH/H ₂ O, 9/1)	Rhodamine	HaCaT (keratinocytes) Human skin tissue	-	MTT (27)	No
2	Hyaluronic acid Sialic acid	Glucuronic acid NANA	Core-shell (QD-MIP)	125	196 μ M (H ₂ O) 64.5 μ M (H ₂ O)	Green QD Red QD	HaCaT KU812 (leukemia)	-	MTT (27)	No
3	Hyaluronic acid	Glucuronic acid	Core-shell (CD-MIP)	184/ +24	-	CD	HeLa (cervical cancer) HaCaT	-	MTT (500)	No
4	Hyaluronic acid	Glucuronic acid	Solid-phase synthesis	70/ +0.13	800 nM (H ₂ O)	Rhodamine	HaCaT	-	-	No
5	Sialic acid Fucose Mannose	Sialic acid Fucose Mannose	Surface imprinting (boronate affinity)	50	200 μ M (phosphate buffer, pH 7.4)	FITC-doped silica	HepG-2 (liver cancer) L-02 (liver cells) MCF-7 (breast cancer) MCF-10A (breast cells)	-	-	Yes
6	Sialic acid Fucose Mannose	Sialic acid Fucose Mannose	Surface imprinting (boronate affinity)	10 (TEM)	-	Yellow QD Red QD Green QD	HepG-2, L-02, MCF-7, MCF-10A HaCaT, HeLa OS-RC-2 (renal cancer) HK-2 (renal cells) SGC-7901 (gastric cancer) HCT-8 (colorectal cancer)	-	-	No
7,8	Sialic acid	Sialic acid	Core-shell (silica -MIP)	~220 (TEM)	1.5 μ M (2 % H ₂ O/98 % CH ₃ OH) 170 μ M (98 % H ₂ O /2 % CH ₃ OH)	Nitrobenzoxadiazole urea monomer	DU145 & PC3 (prostate cancer) Jurkat T (leukemia) HG3, Cl, Wa-osel, AIII (leukemia)	-	-	Yes
9	Sialic acid	Sialic acid	Precipitation polymerization	70	-	Conjugated polymer (2 photon emitter)	4T1 (mouse breast cancer) MCF-10A	4T1 tumor/ -	MTT (100)	No
10	HSA	HSA	Precipitation polymerization	35	-	Fluorescein acrylamide	-	HeLa tumor/ 5	MTT (100)	No
11	EGFR	EEKKVCQGT	Core-shell (CD-MIP)	94/ -43.7	-	CD	HeLa MCF-7	HeLa tumor/ -	CCK-8 (500) H&E: <i>ex-vivo</i> organs	No
12	VEGF	IKPHQGQHI	Solid-phase synthesis	171/ -11.9	1.6 nM (PBS buffer)	QD	-	Zebrafish embryos (VEGF+) (VEGF-)/ >7	Toxicity assays in zebrafish embryos	No

CCK-8, cell counting kit-8 (tetrazolium salt); CD, carbon dot; DLS, dynamic light scattering; EGFR, epidermal growth factor receptor; FITC, fluorescein isothiocyanate; H&E, hematoxylin and eosin; HSA, human serum albumin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NANA, N-acetylneuraminic acid; PBS, phosphate-buffered saline; QD, quantum dot; TEM, transmission electron microscopy; VEGF, vascular epidermal growth factor

Table S1B. MIP-NPs for targeted imaging and therapy

Ref.	Biomarker	Molecular template	Imprinting technique	Size (nm)/ ζ potential (mV)	K_d (solvent)	Fluoroprobe	Cell line	Xenograft/ No. mice	Cell viability assay (MIP _{max} μ g/mL)	Flow cytometry	Therapy
13	EGFR	SLNITSLGLRSLKEISD G & doxorubicin	Solid-phase synthesis	242	7.7 nM to epitope, 3.6 nM to extracellular part of EGFR (PBS buffer)	N-fluorescein acrylamide	MDA-MB-468 MDA-MB-231 SKBR-3 (breast cancers)	-	MTS (30)	Yes	Drug delivery doxorubicin
14	HER2	KDIFHKNNQ & doxorubicin	Core-shell	100	-	Si NP	SKBR-3 BT-474 (breast cancer) MDA-MB-231 HUVEC (umbilical vein)	-	MTT (500)	No	Drug delivery doxorubicin
15	Fn14	Glu-PPAPFRLWP & bleomycin	Core-shell	70 (TEM)	-	Si NP	BxPC-3 (pancreas cancer) A549 (lung cancer) BEAS-2B (lung cells)	BxPC-3 tumor/ 6	CCK-8 (100) H&E: <i>ex vivo</i> organs	No	Drug delivery bleomycin
16	P32	α -helix LHTDGDKAF & doxorubicin	Core-shell	124	-	Si NP	4T1 C8161 (melanoma)	-	CCK-8 (4000) H&E: <i>ex vivo</i> organs	Yes	Drug delivery doxorubicin
17	FR α (NPs decorated with folate ligand)	Vinblastine	Surfactant precipitation polymerization	258/ -2.11	-	Fluorescein (<i>in vitro</i>) Cy7 (<i>in vivo</i>)	HeLa A549	HeLa tumor / 6	MTT (12) H&E: <i>ex vivo</i> organs	Yes	Drug delivery vinblastine
18	Sialic acid	Sialic acid	Surface imprinting (boronate affinity)	40 (length) x 10 (width)	-	Fluorescein (<i>in vitro</i>) NIR 797 (<i>in vivo</i>)	HepG-2 L-02	HepG-2 tumor/ -	MTT (150)	Yes	Photothermal gold nanorods
19	P32	scaffold-based CNCKAPETADCAFVC FLS	Inverse microemulsion polymerization	37	IC ₅₀ (peptide) 340 nM (PBS buffer)	6-aminofluorescein (<i>in vitro</i>) NIR 783 (<i>in vivo</i>)	4T1 BxpC-3	4T1 tumor/ 6	MTT (4000) H&E: <i>ex vivo</i> organs	Yes	Photodynamic methylene blue
20	FR α	QTRIAWARTELLNVA MNAKH	Precipitation polymerization	78	5.7 nM to FR α (PBS buffer)	Nile red (<i>in vitro</i>) NIR 783 (<i>in vivo</i>)	HeLa A549	HeLa tumor/ 6	MTT (20000) H&E: <i>ex vivo</i> organs	Yes	Photodynamic methylene blue
21	Fn14	helical peptide ILGGALSLTFVLGLLSG FLWVRR	Precipitation Polymerization	45	-	Nile red (<i>in vitro</i>) NIR 783 (<i>in vivo</i>)	BxPC-3 BT-474	BxPC-3 tumor/ 6	MTT (8000) H&E: <i>ex vivo</i> organs	Yes	Photodynamic hypocrellin B
22	CD59	YNCNPNTADCK & doxorubicin	Core-shell	84 (TEM)/ +5	-	Gd-doped silicon QD	MCF-7 LoVo (colon cancer)	MCF-7 tumor/ 5	CCK-8 (1000) H&E: <i>ex vivo</i> organs	Yes	Photodynamic Ce6 & drug delivery doxorubicin

CCK-8, cell counting kit-8 (tetrazolium salt); EGFR, epidermal growth factor receptor; Fn14, fibroblast growth factor-inducible 14; FR α , folate-receptor- FR α ; H&E, hematoxylin and eosin; HER2, human epidermal growth factor 2; MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); NIR, near infrared; NP, nanoparticle; PBS, phosphate-buffered saline; QD, quantum dot; Si NP, silicon nanoparticle; TEM, transmission electron microscopy

Table S1C. MIP-NPs as therapeutics

Ref.	Biomarker	Molecular template	Imprinting technique	Size (nm)/ ζ potential (mV)	K_d (solvent)	Fluoroprobe	Cell line	Xenograft/ No. mice	Cell viability assay (MIP _{max} μ g/mL)	Flow cytometry	Therapy
23	N- and E-cadherin	DWVIPPI	Solid-phase synthesis	47	IC ₅₀ (peptide) 5 nM IC ₅₀ (E-cad) 2.5 nM IC ₅₀ (N-cad) 20 nM (phosphate buffer, pH 7.0)	Rhodamine	HaCaT MCF-7 HeLa L929 (murine fibroblast)	-	MTT (250)	No	MIPs inhibit cell-cell adhesion, disrupt spheroids and reduce tumor invasion
24	HER2	N-glycans	Surface imprinting (boronate affinity)	70	-	FITC-doped silica (<i>in vitro</i>) NIR 797-doped silica (<i>in vivo</i>)	SKBR-3 MCF-7	SKBR-3 tumor/ 3-5	MTT (400)	Yes	MIPs inhibit cell proliferation and tumor growth

References

- (1) Kunath, S.; Panagiotopoulou, M.; Maximilien, J.; Marchyk, N.; Sänger, J.; Haupt, K. Cell and Tissue Imaging with Molecularly Imprinted Polymers as Plastic Antibody Mimics. *Adv. Healthc. Mater.* **2015**, *4*, 1322–1326.
- (2) Panagiotopoulou, M.; Salinas, Y.; Beyazit, S.; Kunath, S.; Duma, L.; Prost, E.; Mayes, A. G.; Resmini, M.; Tse Sum Bui, B.; Haupt, K. Molecularly Imprinted Polymer Coated Quantum Dots for Multiplexed Cell Targeting and Imaging. *Angew. Chem. Int. Ed.* **2016**, *55*, 8244–8248.
- (3) Demir, B.; Lemberger, M. M.; Panagiotopoulou, M.; Medina Rangel, P. X.; Timur, S.; Hirsch, T.; Tse Sum Bui, B.; Wegener, J.; Haupt, K. Tracking Hyaluronan: Molecularly Imprinted Polymer Coated Carbon Dots for Cancer Cell Targeting and Imaging. *ACS Appl. Mater. Interfaces* **2018**, *10*, 3305–3313.
- (4) Medina Rangel, P. X.; Laclef, S.; Xu, J.; Panagiotopoulou, M.; Kovensky, J.; Tse Sum Bui, B.; Haupt, K. Solid-Phase Synthesis of Molecularly Imprinted Polymer Nanolabels : Affinity Tools for Cellular Bioimaging of Glycans. *Sci. Rep.* **2019**, *9*, 3923.
- (5) Wang, S.; Yin, D.; Wang, W.; Shen, X.; Zhu, J. J.; Chen, H. Y.; Liu, Z. Targeting and Imaging of Cancer Cells via Monosaccharide-Imprinted Fluorescent Nanoparticles. *Sci. Rep.* **2016**, *6*, 22757.
- (6) Wang, S.; Wen, Y.; Wang, Y.; Ma, Y.; Liu, Z. Pattern Recognition of Cells via Multiplexed Imaging with Monosaccharide-Imprinted Quantum Dots. *Anal. Chem.* **2017**, *89*, 5646–5662.
- (7) Shinde, S.; El-Schich, Z.; Malakpour, A.; Wan, W.; Dizeyi, N.; Mohammadi, R.; Rurack, K.; Gjörlöf Wingren, A.; Sellergren, B. Sialic Acid-Imprinted Fluorescent Core-Shell Particles for Selective Labeling of Cell Surface Glycans. *J. Am. Chem. Soc.* **2015**, *137*, 13908–13912.
- (8) El-Schich, Z.; Abdullah, M.; Shinde, S.; Dizeyi, N.; Rosén, A.; Sellergren, B.; Wingren, A. G. Different Expression Levels of Glycans on Leukemic Cells—a

- Novel Screening Method with Molecularly Imprinted Polymers (MIP) Targeting Sialic Acid. *Tumor Biol.* **2016**, *37*, 13763–13768.
- (9) Zhang, W.; Kang, J.; Li, P.; Liu, L.; Wang, H.; Tang, B. Two-Photon Fluorescence Imaging of Sialylated Glycans *in Vivo* Based on a Sialic Acid Imprinted Conjugated Polymer Nanoprobe. *Chem. Commun.* **2016**, *52*, 13991–13994.
- (10) Takeuchi, T.; Kitayama, Y.; Sasao, R.; Yamada, T.; Toh, K.; Matsumoto, Y.; Kataoka, K. Molecularly Imprinted Nanogels Acquire Stealth *in Situ* by Cloaking Themselves with Native Dysopsonic Proteins. *Angew. Chem. Int. Ed.* **2017**, *56*, 7088–7092.
- (11) Zhang, Y.; Li, S.; Ma, X.; He, X.; Li, W.; Zhang, Y. Carbon Dots-Embedded Epitope Imprinted Polymer for Targeted Fluorescence Imaging of Cervical Cancer via Recognition of Epidermal Growth Factor Receptor. *Microchim. Acta* **2020**, *187*:228.
- (12) Cecchini, A.; Raffa, V.; Canfarotta, F.; Signore, G.; Piletsky, S.; MacDonald, M. P.; Cuschieri, A. *In Vivo* Recognition of Human Vascular Endothelial Growth Factor by Molecularly Imprinted Polymers. *Nano Lett.* **2017**, *17*, 2307–2312.
- (13) Canfarotta, F.; Lezina, L.; Guerreiro, A.; Czulak, J.; Petukhov, A.; Daks, A.; Smolinska-Kempisty, K.; Poma, A.; Piletsky, S.; Barlev, N. A. Specific Drug Delivery to Cancer Cells with Double-Imprinted Nanoparticles against Epidermal Growth Factor Receptor. *Nano Lett.* **2018**, *18*, 4641–4646.
- (14) Wang, H.; Cao, P.; He, Z.; He, X.; Li, W.; Li, Y.; Zhang, Y.-K. Targeted Imaging and Targeted Therapy of Breast Cancer Cells via Fluorescence Double Template-Imprinted Polymer Coated Silicon Nanoparticles by Epitope Approach. *Nanoscale* **2019**, *11*, 17018–17030.
- (15) Jia, C.; Zhang, M.; Zhang, Y.; Ma, Z.-B.; Xiao, N.; He, X.; Li, W.; Zhang, Y. Preparation of Dual-Template Epitope Imprinted Polymers for Targeted Fluorescence Imaging and Targeted Drug Delivery to Pancreatic Cancer BxPC-3 Cells. *ACS Appl. Mater. Interfaces* **2019**, *11*, 32431–32440.
- (16) Qin, Y.; Peng, H.; He, X.; Li, W.; Zhang, Y. Highly Effective Drug Delivery and Cell Imaging Using Fluorescent Double-Imprinted Nanoparticles by Targeting Recognition of the Epitope of Membrane Protein. *Anal. Chem.* **2019**, *91*, 12696–12703.
- (17) Zhu, Y.; Liu, R.; Huang, H.; Zhu, Q. Vinblastine-Loaded Nanoparticles with Enhanced Tumor-Targeting Efficiency and Decreasing Toxicity : Developed by One-Step Molecular Imprinting Process. *Mol. Pharm.* **2019**, *16*, 2675–2689.
- (18) Yin, D.; Li, X.; Ma, Y.; Liu, Z. Targeted Cancer Imaging and Photothermal Therapy via Monosaccharide-Imprinted Gold Nanorods. *Chem. Commun.* **2017**, *53*, 6716–6719.
- (19) Zhang, Y.; Deng, C.; Liu, S.; Wu, J.; Chen, Z.; Li, C.; Lu, W. Active Targeting of Tumors through Conformational Epitope Imprinting. *Angew. Chem. Int. Ed.* **2015**, *54*, 5157–5160.
- (20) Liu, S.; Bi, Q.; Long, Y.; Li, Z.; Bhattacharyya, S.; Li, C. Inducible Epitope Imprinting: “Generating” the Required Binding Site in Membrane Receptors for Targeted Drug Delivery. *Nanoscale* **2017**, *9*, 5394–5397.
- (21) Peng, S.; Wang, Y.; Li, N.; Li, C. Enhanced Cellular Uptake and Tumor Penetration of Nanoparticles by Imprinting the “Hidden” Part of Membrane Receptors for Targeted Drug Delivery. *Chem. Commun.* **2017**, *53*, 11114–11117.

- (22) Peng, H.; Qin, Y.; He, X.; Li, W.; Zhang, Y. Epitope Molecularly Imprinted Polymer Nanoparticles for Chemo-/ Photodynamic Synergistic Cancer Therapy Guided by Targeted Fluorescence Imaging. *ACS Appl. Mater. Interfaces* **2020**, *12*, 13360–13370.
- (23) Medina Rangel, P. X.; Moroni, E.; Merlier, F.; Gheber, L. A.; Vago, R.; Tse Sum Bui, B.; Haupt, K. Chemical Antibody Mimics Inhibit Cadherin-Mediated Cell-Cell Adhesion: A Promising Strategy for Cancer Therapy. *Angew. Chem. Int. Ed.* **2020**, *59*, 2816–2822.
- (24) Dong, Y.; Li, W.; Gu, Z.; Xing, R.; Ma, Y.; Zhang, Q.; Liu, Z. Inhibition of HER2-Positive Breast Cancer Growth by Blocking the HER2 Signaling Pathway with HER2-Glycan-Imprinted Nanoparticles. *Angew. Chem. Int. Ed.* **2019**, *58*, 10621–10625.