A Journal of the Gesellschaft Deutscher Chemiker

Angewandte International Edition

GDCh

Chemie

www.angewandte.org

Accepted Article

Title: Remarkable Effect of Halogen Substitution on the Membrane Transport of Fluorescent Molecules in Living Cells

Authors: Harinarayana Ungati, Vijayakumar Govindaraj, and Govindasamy Mugesh

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201804128
Angew. Chem. 10.1002/ange.201804128

Link to VoR: http://dx.doi.org/10.1002/anie.201804128
http://dx.doi.org/10.1002/ange.201804128

WILEY-VCH

10.1002/anie.201804128

WILEY-VCH

Remarkable Effect of Halogen Substitution on the Membrane Transport of Fluorescent Molecules in Living Cells

Harinarayana Ungati, Vijayakumar Govindaraj and Govindasamy Mugesh*

Abstract: *Small-molecule-based fluorescent probes have become important tools in biology for sensing and imaging applications. However, the biological applications of many of the fluorescent molecules are hampered by low cellular uptake and high toxicity. In this paper, we show for the first time that the introduction of halogen atoms enhances the cellular uptake of fluorescent molecules and the nature of halogen atoms plays a crucial role in the plasma membrane transport in mammalian cells. The remarkably higher uptake of iodinated compounds as compared to that of their chloro or bromo analogues suggests that the strong halogen bonding ability of iodine atoms may play an important role in the membrane transport. This study provides a novel strategy for the transport of fluorescent molecules across the plasma membrane in living cells.*

Fluorescence imaging has emerged as a powerful tool to study the concentration, localization and functions of various biomolecules at the cellular level.[1] Particularly, small-molecule-based fluorescent probes have found diverse applications in the areas of cell biology, drug discovery, chemical biology, and clinical diagnosis.[2] Although a large number of small-molecular fluorescent probes have been developed for various cellular applications,[3-5] the mechanisms by which these molecules enter the cells are not well understood in many cases. Fluorescence probes based on 4-amino-1,8-naphthalimide have attracted significant attention as these compounds can be synthesized in high yields from readily available starting materials and their solubility and fluorescence property can be altered by substitution at the amino and imide moieties (Figure 1).[6] The two-photon fluorescent probe for the detection of formaldehyde[7], the lysosome-targetable probe for monitoring the endogenous nitric oxide (NO),[8] the solvatochromic fluorophore for the development of a protein-based pH sensor,[9] the ratiometric fluorescent probe for monitoring the mitochondrial NO level,[10] the pantetheine probe for the visualization of the chain-flipping mechanism in fatty-acid biosynthesis[11] represent a few selected examples of fluorescent probes that have been employed successfully for cellular applications. In this paper, we report for the first time that a simple substitution of hydrogen atoms with halogens remarkably enhances the cellular uptake of naphthalimide-based fluorescent molecules in mammalian cells. This study also suggests that halogen bonding may facilitate the active transport of halogenated compounds, particularly iodinated compounds, across the plasma membrane.

[*] H. Ungati, Dr. V. Govindaraj and Prof. Dr. G. Mugesh
Department of Inorganic and Physical Chemistry
Indian Institute of Science
Bangalore 560012, India
E-mail: mugesh@iisc.ac.in

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/anie.2018xxxx.

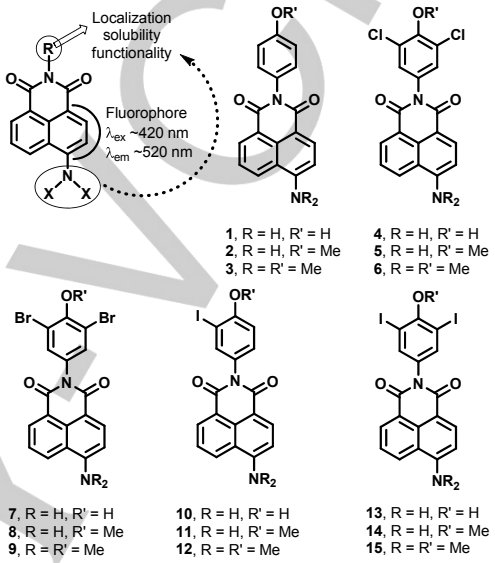
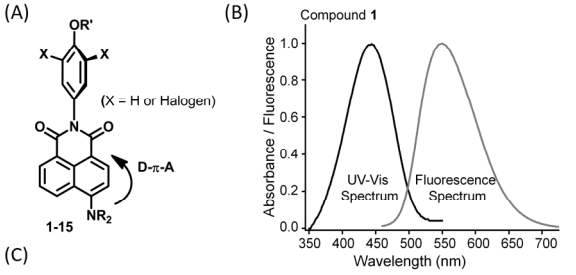


Figure 1. Chemical structure of fluorescent molecules **1**-**15** synthesized in this study based on 4-amino-1,8-naphthalimide moiety.

The naphthalimide-based compounds **1**-**15** (Figure 1) required for this study were synthesized from 4-nitro-1,8-naphthalic anhydride and 4-aminophenol or halogen-substituted 4-aminophenols as described in the Supporting Information. The fluorescent properties of compounds **1-15** were studied in phosphate-buffered saline (PBS) at room temperature. As these compounds have low solubility in an aqueous medium, the compounds were dissolved in DMSO and diluted with PBS buffer by 1:100. The luminescent properties of compounds **1-15** strongly depend on the substituents at the naphthalimide and phenolic rings with the $-NR_2$ and substituted phenyl group acting as the electron donor and acceptor, respectively. As expected, compound **1** exhibited a significant fluorescence by push-pull mechanism (Figure 2A-D) and there was an enhancement in the fluorescence when the strongly electron donating –OH group at the phenyl ring was replaced by a less electron-donating methoxy group (compound **2**). A further increase in the fluorescence intensity was observed when an $-NMe_2$ group was introduced at the 4-position of the naphthalimide moiety (compound **3**). A similar trend was observed when halogen atoms were introduced to the phenolic ring at 2,6-positions. However, a gradual decrease in the fluorescence intensity was observed upon introduction of chlorine, bromine and iodine atoms (Figure 2D), which is in agreement with the heavy-atom effect observed for other related fluorescent systems.[12] To understand the lipophilicity of compounds **1-15**, the partition coefficient (P) in octanol-water biphasic system was determined

by using HPLC and compared the experimental log P values with the corresponding theoretical values obtained using the ALOGPS program (Figure 2E).[13] These results indicate that there is no major change in the lipophilicity upon introduction of heavier halogen atoms.



(C)

Compd.	λ_{ex} nm	λ_{em} nm	Compd.	λ_{ex} nm	λ_{em} nm	Compd.	λ_{ex} nm	λ_{em} nm
1	434	546	6	424	554	11	438	541
2	434	546	7	434	546	12	428	552
3	434	555	8	428	535	13	434	548
4	434	550	9	434	560	14	442	555
5	434	555	10	434	546	15	434	558

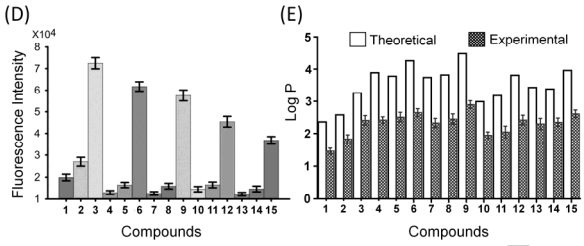


Figure 2. (A) The D-π-A interactions that are responsible for the fluorescence bahaviour of compounds **1-15**. (B) The normalized UV-Vis and fluorescence spectra of compound **1** in water. (C) Excitation and emission wavelengths for compounds **1-15**. (D) The relative fluorescence intensity for compounds **1-15** in phosphate-buffered saline (PBS). (E) The lipophilicity of compounds **1-15** given in terms of their log P values.

The interesting fluorescent properties of compounds **1-15** prompted us to investigate their uptake in mammalian cells. We studied the cellular uptake of the fluorescent molecules in HepG2 (human liver carcinoma), HEK293 (human embryonic kidney) cells by using laser scanning microscopy and fluorescence microplate reader techniques. The HepG2 cells treated with compounds **1**-**3** for 30 min at 10 μM concentration showed a very low fluorescence (Figure 3A and 3B), indicating that these compounds are not taken up readily by the cells. When the cell culture medium was analysed after 30 min of treatment, it was observed that only small amounts of compounds **1** (5%), **2** (6%) and **3** (8%) entered the cells (Figure 3B, S47-49). The cellular uptake was marginally improved for compounds **4** (7%), **5** (10%) and **6** (15%), suggesting that the introduction of chlorine atoms increases the cellular uptake. Furthermore, the higher uptake of compound **9** (22%) having two bromine atoms and an –NMe_2 group indicates that the introduction of heavier halogen may facilitate the cellular entry of the 1,8-naphthalimide derivatives. Interestingly, the introduction of an iodine atom significantly increased the cellular uptake as the amounts of compounds **10**, **11** and **12** entered the cells were determined to be 14%, 22% and 38%, respectively (Figure 3B). Remarkably, a rapid cellular uptake was observed for the diiodo compounds **13** (22%), **14** (41%) and **15** (98%), indicating that the steric hindrance introduced by two iodine atoms does not block the transport of these compounds across the plasma membrane. A similar trend was observed when HEK293 and HeLa cells were used for the experiments (Figure S52-53). To understand whether the heavier halogen atoms can facilitate the uptake in normal cells, we studied the uptake of compounds **1-15** in primary endothelial cells (HUVEC) isolated from human umbilical vein. A facile uptake of compounds **12** and **15** was observed in these cells as well (Figure 3A, S50).

As substitution of heavier halogen may lead to higher toxicity, the cell viability was determined by using the standard MTT assay. Interestingly, the toxicity of the bromo and iodo compounds (**7**-**15**) was found to be almost identical to that of the unsubstituted compounds **1**-**3** (Figure 3C), indicating that the

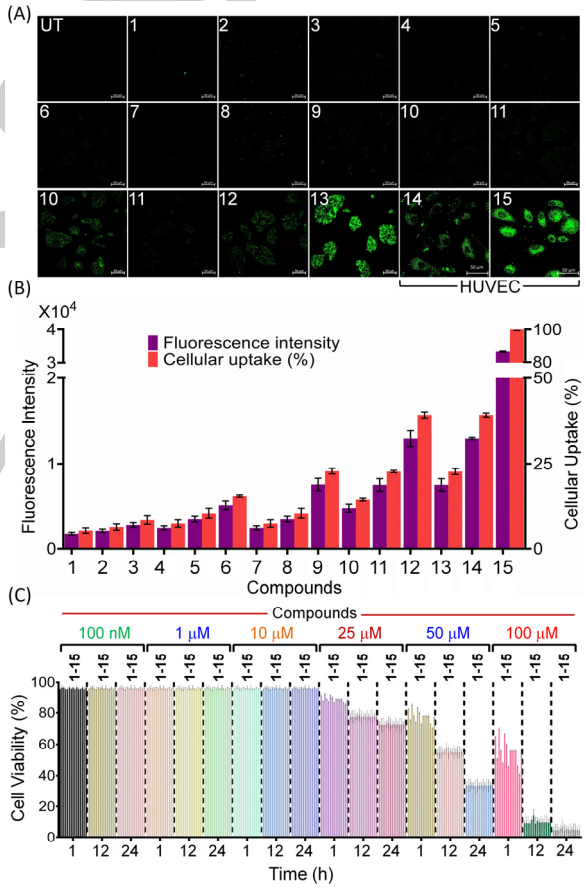


Figure 3. (A) Confocal microscopy images of HepG2 cells treated with compounds **1-15** (10 μM) for 30 min. The last two images indicate the uptake of the two iodo compounds **12** and **15** by human umbilical vein endothelial cells (HUVEC, primary cells) under identical conditions. (B) The fluorescence measured by a plate reader after 30 min of treatment of HepG2 cells with compounds **1-15** (10 μM) and the total uptake of compounds **1-15** by HepG2 cells. The % cellular uptake was calculated from the amount of compounds left in the medium after 30 min treatment. (C) Cell viability of HepG2 cells incubated with compounds **1-15** at various concentrations after 1, 12 and 24h.

introduction of heavier halogen atoms does not alter the toxicity of the compounds. While the compounds did not exhibit any cytotoxicity up to 10 μM concentration, they were found to be toxic to the cells at 25 μM and above concentrations. Recently, Yang and co-workers reported a biocompatible halogen-containing fluorescent probe (HKOH-1r) based on fluorescein for the detection of endogenous hydroxyl radicals in living cells.[4c] In this work, the authors demonstrated that the diiodophenol acts as a quencher of fluorescein or 2′,7′-dichlorofluorescein and the steric hindrance of the two iodine atoms blocks the oxidation by strong oxidants such as HOCl or peroxynitrite. However, it is not clear whether the iodine atoms play any role in the cellular uptake of the fluorescent probes.

The remarkably higher uptake of the diiodo compound **15** (98% in HepG2 cells) as compared to other compounds indicates that the iodo compounds may be transported across the plasma membrane by specific transporters that have high affinity for iodine atoms. It is known that the iodine-containing thyroid hormone, thyroxine (T4, Figure 4A), crosses the plasma membrane with the help of transport proteins. Although T4 and its metabolites are hydrophobic in nature, they cannot simply diffuse across the lipid bilayer membrane, probably due to the larger size of iodine atoms and the charged nature of the amino acid moiety. Visser and co-workers reported that the monocarboxylate transporter 8 (MCT8) acts as a very active and specific thyroid hormone transporter.[14] It is known that mutations in the human MCT8 leads to a genetic disorder known as Allan–Herndon–Dudley syndrome.[15] Therefore, it was thought worthwhile to understand the mechanism of transport of compound **15** in mammalian cells. Interestingly, when HepG2 cells were co-treated with compound **15** (10 μM) and T4, it was found that T4 inhibits the uptake of compound **15** in a dose-dependent manner (Figure 4A and 4B). At 1 μM concentration of T4, an almost 70% inhibition of the cellular uptake of compound **15** was observed. To understand whether the number of iodine atoms plays an important role, the cellular uptake of **15** was studied in the presence of T3, 3,5-T2 and T1, having three, two and one iodine atom(s), respectively. While T3 blocks the cellular uptake of **15** as efficient as T4, a significantly decreased inhibition was observed in the presence of T2. In contrast, T1 having one iodine atom was unable to block the uptake of **15** under identical conditions. Similarly, no inhibition was observed for the fully deiodinated thyroxine derivative T0 even at 10 μM concentration (Figure 4A). These observations indicate that the fluorescent molecules **1-15**, particularly the halogenated compounds, enter the cells through the thyroid hormone transporter and the higher uptake of compound **15** is due to the presence of two iodine atoms that appear to play a crucial role in the membrane transport.

It has been shown by Johannes *et al* that silychristin, a flavonolignan derived from the milk thistle, is a potent inhibitor of MCT8.[16] When the cellular uptake of compound **15** was carried out in HepG2 cells, silychristin strongly inhibited the uptake in a dose-dependent manner with an IC_{50} value of 8.13 μM, confirming that MCT8 is involved in the transport of the halogen-substituted fluorescent molecules. An almost complete inhibition of cellular uptake was observed at higher concentrations of silychristin (Figure 4D and S56), which indicates that transporters other than MCT8 are probably not involved in the transport and the introduction of heavier halogen may increase the selectivity of transport through a biological membrane. The inhibition of membrane transport of compound **15** by silychristin was also observed in HeLa and HEK293 cells (Figure S57 and S58). A similar transport is expected to occur in HUVEC as MCT8 is the major transporter of T4 and T3 in all mammalian cells.

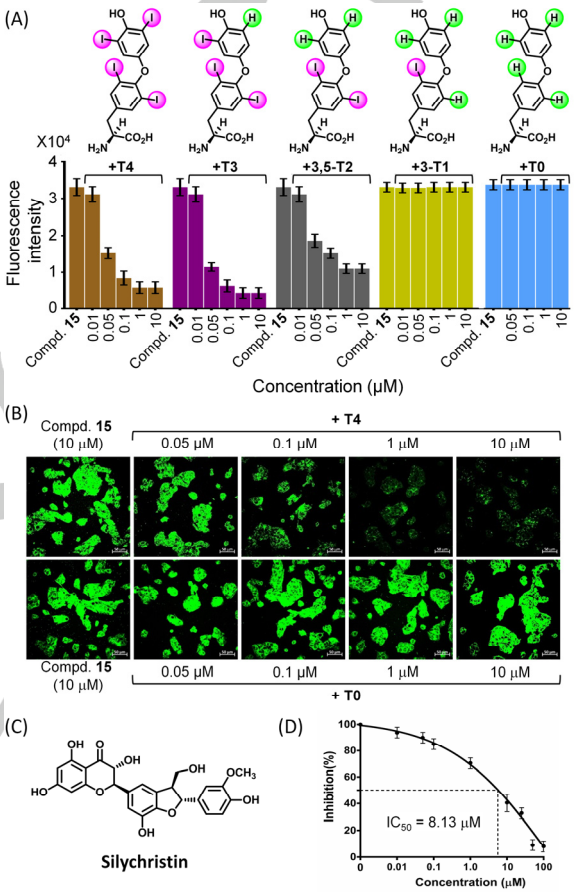


Figure 4. (A) The fluorescence measured by a plate reader after 30 min of treatment of HepG2 cells with compound **15** (10 μM) in the presence of various concentrations (0.01 - 10.0 μM) of thyroxine (T4) and its metabolites T3, 3,5-T2, 3-T1 and T0. (B) Confocal microscopy images of HepG2 cells co-treated with compound **15** (10 μM) and various concentrations of T4 or T0 for 30 min. (C) Chemical structure of silychristin, a potent inhibitor of the thyroid hormone transporter MCT8. (D) Inhibition of the cellular uptake of compound **15** by silychristin. The fluorescence was measured by a plate reader after treating the HepG2 cells with various concentrations of silychristin for 20 min, followed by treatment with compound **15** (10 μM) for 20 min.

For each series of compounds **4-6**, **7-9**, **10-12** and **13-15** (Figure 1), the cellular uptake increases with an increase in the size of the halogen atom. For example, the cellular entry of compounds **6**, **9**, **12** and **15**, is higher than that of the unsubstituted compound **3**, and the uptake follows the order I > Br > Cl. For the iodo compounds **10**-**15**, the internalization of the

diiodo compounds **13-15** is found to be higher than that of the corresponding monoiodo compounds (**10**-**12**). It is known that halogen atoms form halogen bonds with electron donors and their halogen bonding ability increases from chlorine to bromine to iodine.[17] Owing to the anisotropic distribution of charge on halogen (X) atoms, a positively charged electrostatic region is created along the C−X bonds. The region of positive electrostatic potential, known as σ-hole, can interact attractively with the electron donors, forming halogen bonds.[18] Although halogen bonds has attracted significant attention in drug discovery,[19] it remains unclear whether they play key roles beyond their applications in improving drug−target interactions. Matile and co-workers and others showed that halogen bonds can be used for ion transport in synthetic membrane systems.[20]

Although the thyroid hormones, T4 and T3, are known to form halogen bonds with their transport proteins transthyretin (TTR) and thyroxine-binding globulin (TBG),[21] it is not known whether halogen bonding is responsible for the cellular uptake of thyroid hormones by MCT8. The present study indicates that halogen bonds may play crucial roles in cellular uptake of organohalogen compounds. The density functional theory (DFT)[22] calculations on the halogenated compounds **4**-**15** indicate that the halogen bonding ability of the iodo compounds is greater than that of the corresponding bromo or chloro compounds. A comparison of the positive electrostatic potential (σ-hole), calculated by natural bond orbital (NBO) analysis,[23] indicates that the σ-hole on the iodine atoms of compound **12** is less positive than that of **15** (Figure 5A), indicating that the removal of one iodine from **15** decreases the positive potential on the other iodine atom. Similarly, a significant decrease in the positive potential was observed for compounds **6** and **9**, which have chlorine and bromine atoms, respectively. Interestingly, the cellular uptake of these compounds follows the same order, i.e. **15** > **12** > **9** > **6**. As T4 and T3 having four and three iodine atoms, respectively, can strongly bind to the receptor, these compounds were able to compete with compound **15** for the cellular entry at low concentrations. It should be noted that the experimentally determined Log P coefficients for T4 (0.85) and T3 (0.52) are much lower than that of **15** (2.61), suggesting that the lipophilicity of the compounds does not play any major role in the cellular uptake. To understand whether the introduction of heavier halogen can be used as a general strategy for improving the cellular uptake of fluorescent molecules, we synthesized compounds **16**-**23** (Figure 5B) and studied their fluorescent behaviour. Interestingly, the cellular uptake of the iodinated compounds **22** and **23** was found to be much higher than that of **16**-**21** (Figure 5D), although compounds **22** and **23** are intrinsically less fluorescent than the corresponding unsubstituted compounds **16** and **17** (Figure 5C). It is worthwhile to note that several halogenated compounds that are commonly used in food packaging and as flame retardants are known to disrupt the endocrine function, but the mechanism by which these compounds are transported into the cells is not clear.[24]

In summary, we showed for the first time that the introduction of iodine atom to a series of naphthalimide-based fluorescent molecules remarkably enhances their cellular uptake by increasing the active transport across the plasma membrane in

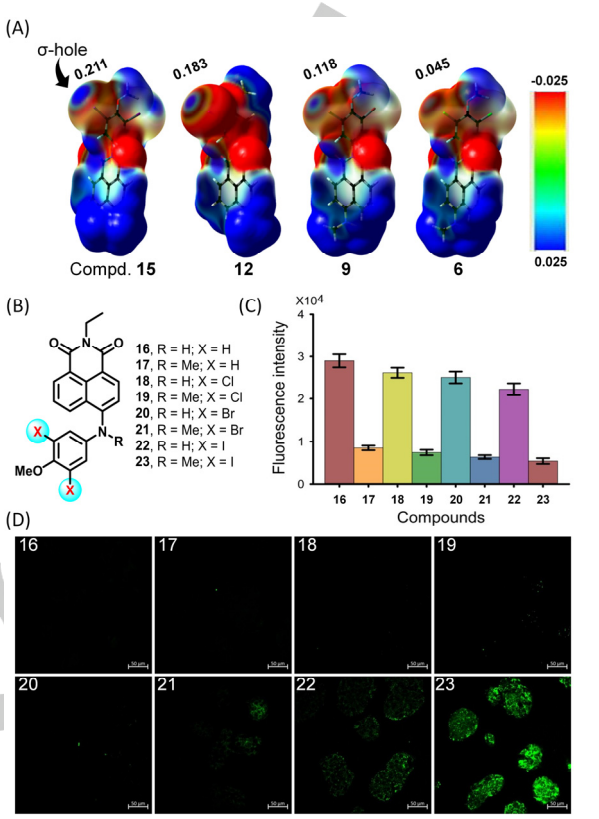


Figure 5. (A) Charges obtained from natural bond orbital (NBO) analysis for compounds **15**, **12**, **9** and **6**. (B) Chemical structures of compounds **16**-**23**. (C) The relative fluorescence intensity for compounds **16**-**23** in phosphate-buffered saline (PBS). (D) Confocal microscopy images of HepG2 cells treated with compounds **16**-**23** (10 μM) for 30 min.

living cells. Several competitive experiments with thyroxine (T4) and its metabolites and with a specific inhibitor, sylichristin, indicate that the organohalogen compounds are transported into the mammalian cells (HepG2, HeLa and HEK293) by the thyroid hormone-specific monocarboxylate transporter 8 (MCT8). Halogen bonding appears to pay crucial roles in the membrane transport and the introduction of heavier halogens may provide a novel strategy to improve the cellular uptake of organic molecules for studying membrane activity, cellular functions, and drug delivery. Further studies are in progress in our laboratory to understand the interaction of MCT8 and related transporters with thyroid hormones and their metabolites.

Acknowledgements

This study was supported by the Science and Engineering Research Board (SERB, EMR/IISc-01/2016), Department of Science and Technology (DST), New Delhi. H. U. and V. G. acknowledge the Indian Institute of Science, Bangalore, and

SERB, respectively, for a research fellowship. We thank Ms Megha Narayanan, Mr Subhash Garhwal and Ms Chithra R. Nair for their help with the synthesis of some of the compounds and Mrs Mary Nirmala Sarkar for her help with the cell culture work. G. M. thanks the SERB for the award of J. C. Bose National fellowship (SB/S2/JCB-067/2015).

Keywords: cellular uptake • halogen • fluorescent molecules • MCT8 transporter • thyroid hormones

[1] a) M. T. Spence, I. D. Johnson, *Molecular probes handbook: a guide to fluorescent probes and labeling technologies*, 11th ed., Life Tech. Corp. , Carlsbad, CA, **2010**; b) E. A. Specht, E. Braselmann, A. E. Palmer, *Annu. Rev. Physiol.* **2017**, *79*, 93-117.

[2] a) M. Schäferling, *Angew. Chem. Int. Ed.* **2012**, *51*, 3532-3554; b) N. Jiang, J. Fan, F. Xu, X. Peng, H. Mu, J. Wang, X. Xiong, *Angew. Chem. Int. Ed.* **2015**, *54*, 2510-2514; c) M. A. Miller, R. Weissleder, *Nat. Rev. Cancer* **2017**, *17*, 399-414; d) K. J. Bruemmer, T. F. Brewer, C. J. Chang, *Curr Opin Chem Biol.* **2017**, *39*, 17-23.

[3] a) A. T. Aron, M. C. Heffern, Z. R. Lonergan, M. N. Vander Wal, B. R. Blank, B. Spangler, Y. Zhang, H. M. Park, A. Stahl, A. R. Renslo, *Proc. Natl. Acad. Sci. U.S.A.* **2017**, *114*, 12669-12674; b) A. Sargenti, G. Farruggia, N. Zaccheroni, C. Marraccini, M. Sgarzi, C. Cappadone, E. Malucelli, A. Procopio, L. Prodi, M. Lombardo, *Nat. Protoc.* **2017**, *12*, 461-471; c) W. Zhou, R. Saran, J. Liu, *Chem. Rev.* **2017**, *117*, 8272-8325; d) Z. Hao, R. Zhu, P. R. Chen, *Curr. Opin. Chem. Biol.* **2018**, *43*, 87-96.

[4] a) Q. Chen, C. Liang, X. Sun, J. Chen, Z. Yang, H. Zhao, L. Feng, Z. Liu, *Proc. Natl. Acad. Sci. U.S.A.* **2017**, *114*, 5343-5348; b) D. Cheng, Y. Pan, L. Wang, Z. Zeng, L. Yuan, X. Zhang, Y.-T. Chang, *J. Am. Chem. Soc.* **2016**, *139*, 285-292; c) X. Bai, Y. Huang, M. Lu, D. Yang, *Angew. Chem. Int. Ed.* **2017**, *56*, 12873-12877; d) J. J. Hu, N.-K. Wong, S. Ye, X. Chen, M.-Y. Lu, A. Q. Zhao, Y. Guo, A. C.-H. Ma, A. Y.-H. Leung, J. Shen, *J. Am. Chem. Soc.* **2015**, *137*, 6837-6843; e) L. Wu, I.-C. Wu, C. C. DuFort, M. A. Carlson, X. Wu, L. Chen, C.-T. Kuo, Y. Qin, J. Yu, S. R. Hingorani, *J. Am. Chem. Soc.* **2017**, *139*, 6911-6918.

[5] S. T. Manjare, Y. Kim, D. G. Churchill, *Acc. Chem. Res.* **2014**, *47*, 2985-2998 and references therein.

[6] a) R. M. Duke, E. B. Veale, F. M. Pfeffer, P. E. Kruger, T. Gunnlaugsson, *Chem. Soc. Rev.* **2010**, *39*, 3936-3953; b) K. G. Leslie, D. Jacquemin, E. J. New, K. A. Jolliffe, *Chem. Eur. J.* **2018**, *24*, 1-6.

[7] Y. Tang, X. Kong, A. Xu, B. Dong, W. Lin, *Angew. Chem. Int. Ed.* **2016**, *55*, 3356-3359.

[8] H. Yu, Y. Xiao, L. Jin, *J. Am. Chem. Soc.* **2012**, *134*, 17486-17489.

[9] M. Yang, Y. Song, M. Zhang, S. Lin, Z. Hao, Y. Liang, D. Zhang, P. R. Chen, *Angew. Chem. Int. Ed.* **2012**, *51*, 7674-7679.

[10] X. Zhu, J. Q. Chen, C. Ma, X. Liu, X. P. Cao, H. Zhang, *Analyst* **2017**, *142*, 4623-4628.

[11] J. Beld, H. Cang, M. D. Burkart, *Angew. Chem. Int. Ed.* **2014**, *53*, 14456-14461.

[12] V. Osipov, M. Usacheva, I. Dilung, *Appl. Spectrosc.* **1983**, *39*, 1181-1185.

[13] a) I. V. Tetko, P. Bruneau, *J. Pharm. Sci.* **2004**, *93*, 3103-3110; b) I. V. Tetko, J. Gasteiger, R. Todeschini, A. Mauri, D. Livingstone, P. Ertl, V. A. Palyulin, E. V. Radchenko, N. S. Zefirov, A. S. Makarenko, *J. Comput. Aid. Mol. Des.* **2005**, *19*, 453-463.

[14] G. Hennemann, R. Docter, E. C. Friesema, M. de Jong, E. P. Krenning, T. J. Visser, *Endocr. Rev.* **2001**, *22*, 451-476.

[15] E. C. Friesema, S. Ganguly, A. Abdalla, J. E. M. Fox, A. P. Halestrap, T. J. Visser, *J. Biol. Chem.* **2003**, *278*, 40128-40135.

[16] J. Johannes, R. Jayarama-Naidu, F. Meyer, E. K. Wirth, U. Schweizer, L. Schomburg, J. Köhrle, K. Renko, *Endocrinology* **2016**, *157*, 1694-1701.

[17] P. Metrangolo, F. Meyer, T. Pilati, G. Resnati, G. Terraneo, *Angew. Chem. Int. Ed.* **2008**, *47*, 6114-6127.

[18] P. Politzer, P. Lane, M. C. Concha, Y. Ma, J. S. Murray, *J. Mol. Model.* **2007**, *13*, 305-311.

[19] Y. Lu, Y. Liu, Z. Xu, H. Li, H. Liu, W. Zhu, *Expert Opin. Drug Discov.* **2012**, *7*, 375-383.

[20] a) A. V. Jentzsch, A. Hennig, J. Mareda, S. Matile, *Acc. Chem. Res.* **2013**, *46*, 2791–2800; b) A. V. Jentzsch, S. Matile, *Top. Curr. Chem.* **2015**, *358*, 205-239. c) C. Ren, X. Ding, A. Roy, J. Shen, S. Zhou, F. Chen, S. F. Y. Li, H. Ren, Y. Y. Yang, H. Zeng, *Chem. Sci.* **2018**, DOI: 10.1039/C8SC00602D.

[21] a) T. Eneqvist, E. Lundberg, A. Karlsson, S. Huang, C. R. Santos, D. M. Power, A. E. Sauer-Eriksson, *J. Biol. Chem.* **2004**, *279*, 26411-26416; b) S. Mondal, K. Raja, U. Schweizer, G. Mugesh, *Angew. Chem. Int. Ed.* **2016**, *55*, 7606-7630.

[22] a) C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785; b) A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648-5652; c) M. J. Frisch, *et. al. Gaussian Inc.,* Wallingford CT, **2004**; d) M. J. Frisch, *et. al. Gaussian Inc.,* Wallingford CT, **2009**.

[23] a) E. D Glendening, J. E. Reed, J. E. Carpenter, F. Weinhold, *NBO Program 3.1*; Madison, WI, **1988**; b) A. E. Reed, L. A. Curtiss, F. Weinhold, *Chem. Rev.* **1988**, *88*, 899-926.

[24] E. Diamanti-Kandarakis, J.-P. Bourguignon, L. C. Giudice, R. Hauser, G. S. Prins, A. M. Soto, R. T. Zoeller, A. C. Gore, *Endocr. Rev.* **2009**, *30*, 293-342.

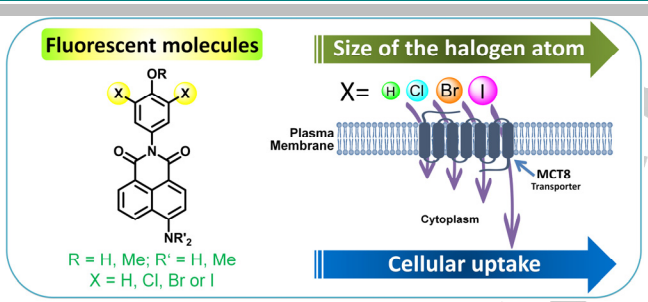
10.1002/anie.201804128

WILEY-VCH

Entry for the Table of Contents

COMMUNICATION

The introduction of halogen atoms enhances the cellular uptake of fluorescent molecules, providing a novel strategy for the transport of organic molecules across the plasma membrane in living cells.



*Harinarayana Ungati, Vijayakumar Govindaraj, Govindasamy Mugesh**

Page No. – Page No.

Remarkable Effect of Halogen Substitution on the Membrane Transport of Fluorescent Molecules in Living Cells