Centre de Recherche en Cancérologie et Immunologie
NANTES ANGERS

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New chemical tools for the production of ^{211}At-labeled radiopharmaceuticals

Dr. François Guérard
CRCI²NA-Nuclear Oncology Team
Nantes-France

^{211}At ecosystem in Nantes

- Arronax cyclotron facility
- CRCI2NA (Cancer research center)
- Subatech (Subatomic physics and technologies) : analytical radiochemistry
- Ceisam : chemistry (molecular modeling)

Why astatine-211?

- One of the eight main α emiters considered for Targeted Radionuclide Therapy

TABLE 1. CHARACTERISTICS OF α-EMITTING RADIONUCLIDES OF POTENTIAL INTEREST FOR THE THERAPY OF CANCERS

Nuclide	*Half-life*	*Decays*	*Energy α (MeV)*	*Production*
^{225}Ac	10 days	4 α, 2 β^-	5.1–8.4	^{233}U decay/cyclotron
^{211}At	7.2 hours	1 α, 1 EC	5.9 or 7.4	Cyclotron
^{212}Bi	61 minutes	1 α, 1 β^-	6.1/7.8	^{228}Th decay/^{224}Ra generator
^{213}Bi	46 minutes	1 α, 2 β^-	6.0/8.4	^{225}Ac generator
^{223}Ra	11.4 days	4 α, 2 β^-	5.7–7.5	^{227}Ac generator
^{149}Tb	4.1 hours	1 α, EC	4.0	Accelerator
^{227}Th	18.7 days	5 α, 2 β^-	5.7–7.5	^{227}Ac generator
^{212}Pb/^{212}Bi[a]	10.6 hours	1 α, 2 β^-	6.1/7.8	^{224}Ra generator

Choice of an α emitter guided by :

- Half-life
- Daughter radionuclides (cascade)
- Chemistry
- Nuclear wastes
- **Availability**

Eychenne et al. Pharmaceutics. 2021, *13*, 906

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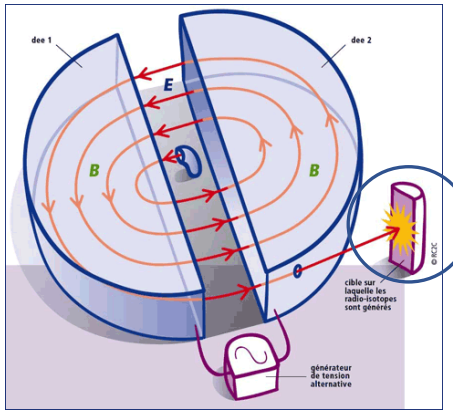
Why astatine-211?

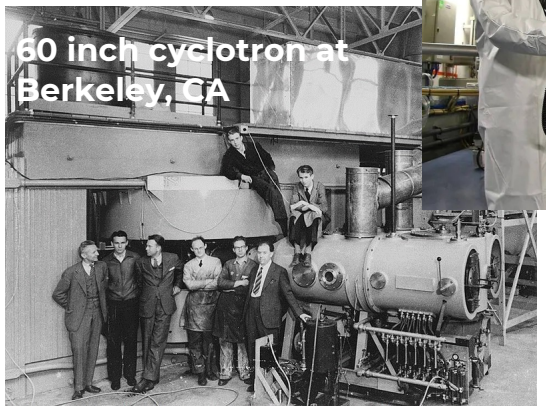
- Increasing worldwide availability



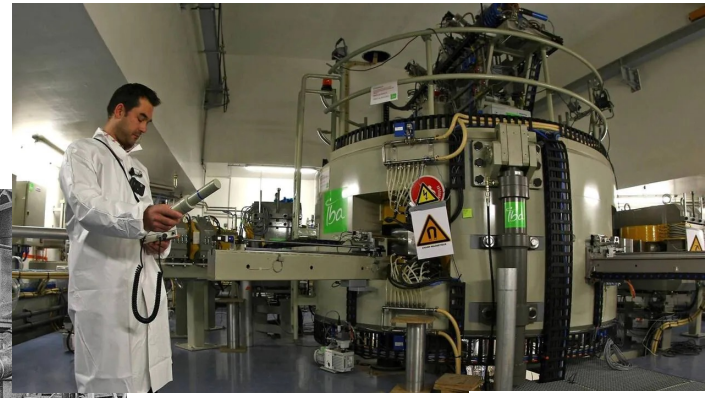
$^{209}Bi + \alpha \rightarrow {}^{211}At + 2n$
(28-29 MeV)

Corson et al. Phys. Rev. 1940, 56, 672

1939



2010

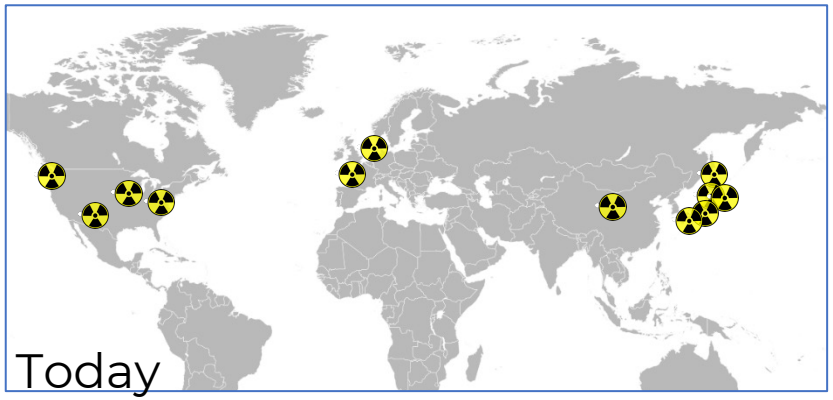


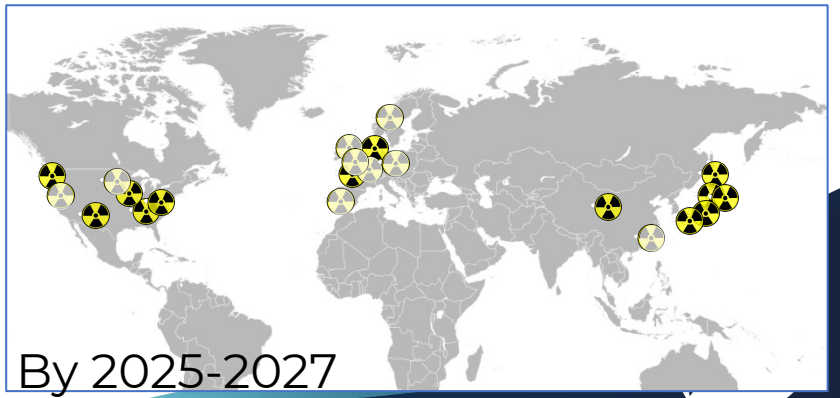
Arronax Nantes

^{211}At production:

Advantage: on demand production from cheap raw material

Drawback: short distribution distance ($t_{1/2}$ = 7.2 h)





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The challenge of astatine chemistry

- No stable isotopes: the most stable (^{210}At) has a short half live of 8.1 h
- The rarest of all natural elements on Earth!
Only ≈ 30 g estimated on Earth.
- It is “invisible” : conventional analysis tools cannot detect such tiny amounts (NMR, IR, UV, mass spectrometry)
- It’s chemistry is often predicted by extrapolation from its closest chemical element, iodine…
- … with however some unexpected observations due to relativistic effects that provide a metallic character

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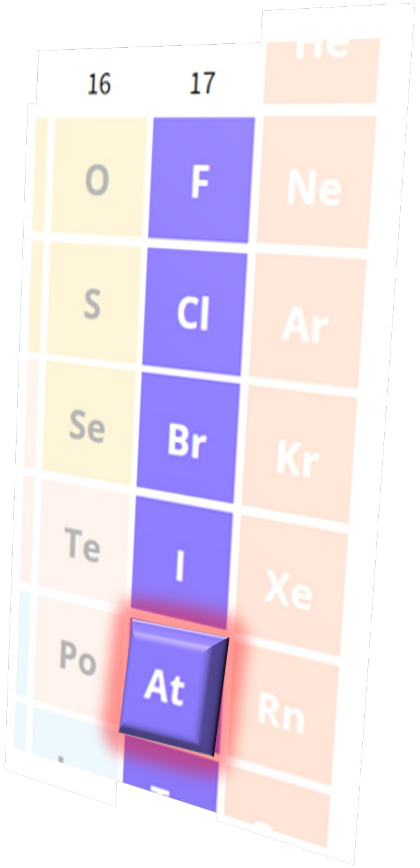
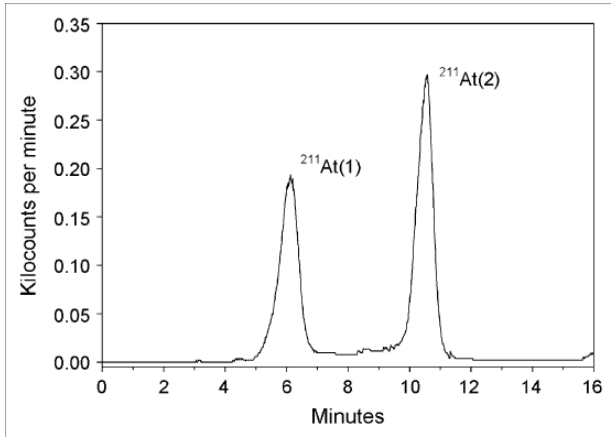
The challenge of astatine chemistry

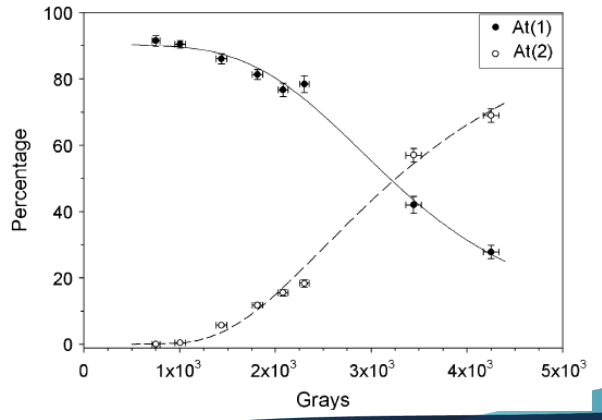
- Reproducible chemistry is a challenge:



➢ impurities are often more concentrated than astatine (picomolar concentration) and uncontrollable side reactions can occur from one astatine batch to another one.

➢ Astatine oxidation state evolves over time due to solvent radiolysis

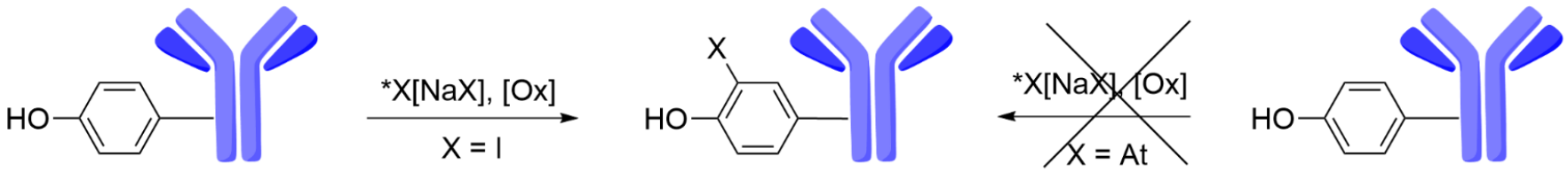




Pozzi et al. J. Nucl. Med. 2007, 48, 1190

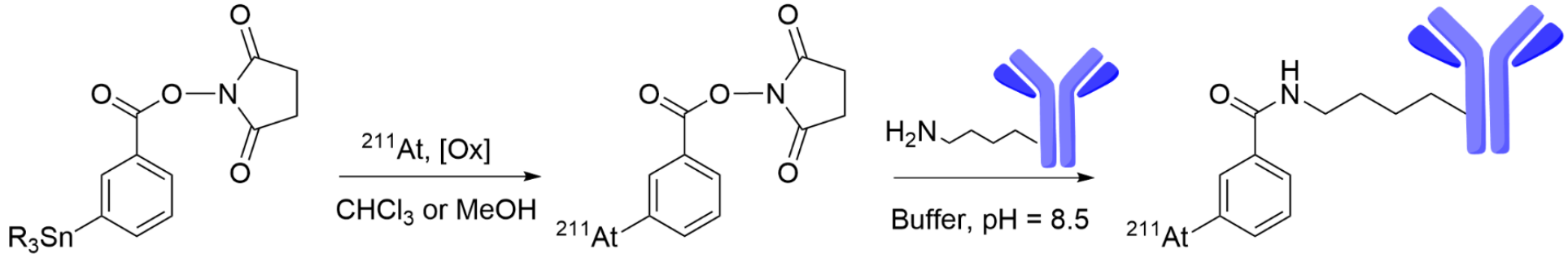
The challenge of astatine chemistry

- Unsuccessful attempts to transpose known radioiodination method



Visser et al, Int. J. Appl. Radiat. Isot. 1981, *32*, 905-912

- 2 step procedure required



[^{211}At]SAB

Zalutsky et al. Proc. Natl. Acad. Sci. U.S.A 1989, *86*, 7149-7153

I-Understanding astatine chemical properties

(Is At a halogen or a metal ?)

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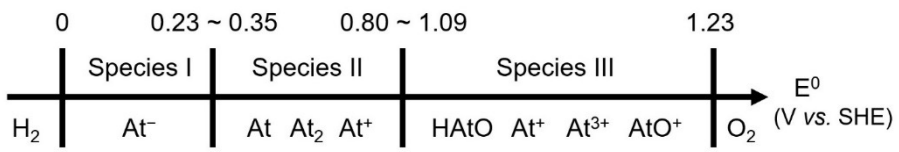
Pourbaix Diagram of Astatine: Evidence of a Metallic Behaviour

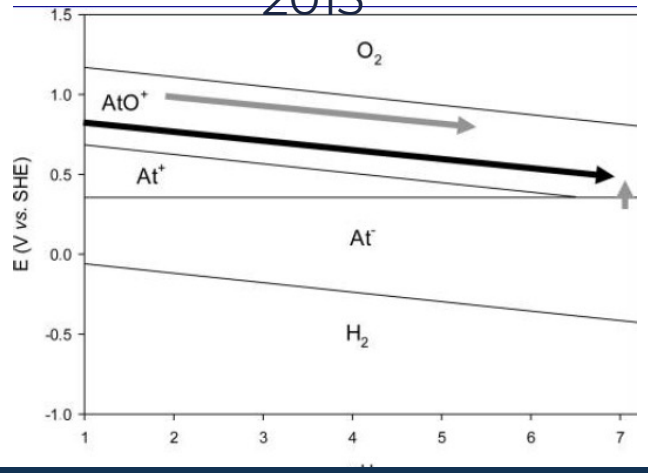
Proposed species at pH = 0, litterature from 1961 to 1989 :



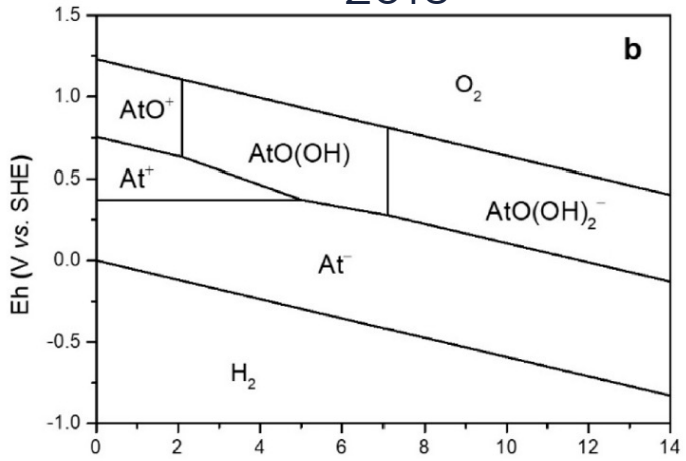
Combination of:

- Ion exchange chromatography
- Electromigration
- Molecular dynamics simulations

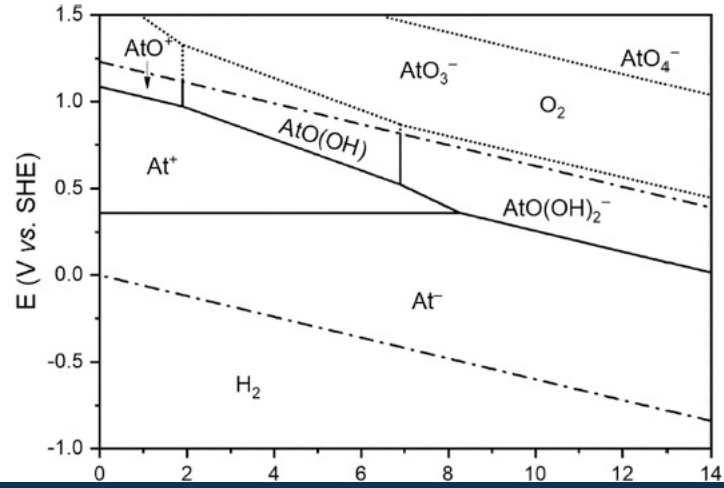
2013



2016



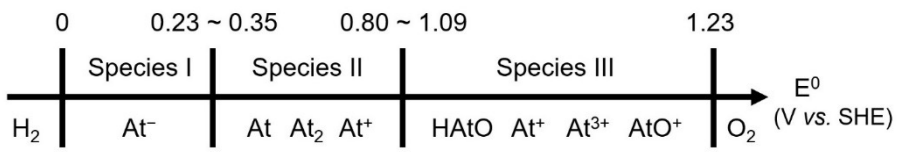
2022



Champion, J., et al. 2009. *Inorganica Chimica Acta* 362 (8): 2654-61. Liu, L. et al. 2022. *Inorg. Chem. 61 (34), 13462–13470*
Sergentu et al. 2016. *Chem. Eur. J* 22 (9): 2964-71

Pourbaix Diagram of Astatine: Evidence of a Metallic Behaviour

Proposed species at pH = 0, litterature from 1961 to 1989 :



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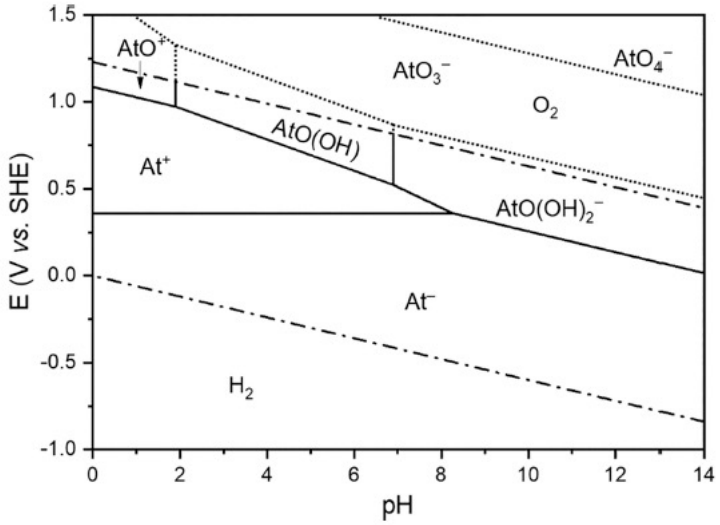

At⁺ stable in water : typical of a metal
Not observed with lighter halogens

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Pourbaix Diagram of Astatine: Evidence of a Metallic Behaviour

- Metallic properties of At+ and AtO+

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Determination of stability constants between complexing agents and At(I) and At(III) species present at ultra-trace concentrations

J. Champion [a], C. Alliot [b], S. Huclier [a], D. Deniaud [c], Z. Asfari [d], G. Montavon [a,*]

Table 1
Parameters describing the interaction between At(*x*)$^+$ and SCN^- and LH_4 using the CMLL and CMSL methodologies.

Ligand	At(I)					At(III)				
	Method used	D/Kd (mL/g)		Complex	*logK*	Method used	D/Kd (mL/g)		Complex	logK
		At(I)	Complex				At(III)	Complex		
LH_4	CMLL (Toluene)	21	–	1:1	4.5 ± 0.4	CMLL (Toluene)	42.5	–	1:1	3.3 ± 0.3
	CMLL (Chloroform)	8	–			CMLL (Chloroform)	2.8	–		
	CMSL	90.4	–			CMSL	28.4	–		
SCN^-	CMLL (Toluene)	144	–	1:2	5.9 ± 0.3	CMLL (Toluene)	60	–	1:2	5.3 ± 0.2
	CMLL (hexane)	3.4	–	1:1	3.8 ± 0.2	CMLL (hexane)	0.95	–	1:1	2.8 ± 0.2
	CMSL	268	10.4 (cpx 1:2)			CMSL	47.7	22 (cpx 1:2)		

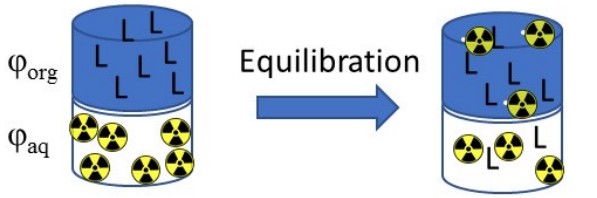
PRL **111,** 116404 (2013) PHYSICAL REVIEW LETTERS week ending 13 SEPTEMBER 2013

Condensed Astatine: Monatomic and Metallic

Andreas Hermann

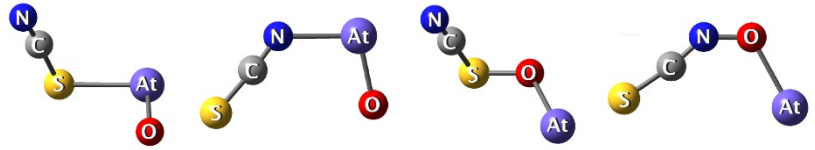
School of Physics and Astronomy and Centre for Science at Extreme Conditions, University of Edinburgh, Edinburgh, EH9 3JZ, United Kingdom

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853, USA



determination of Kd

Molecular modelling:

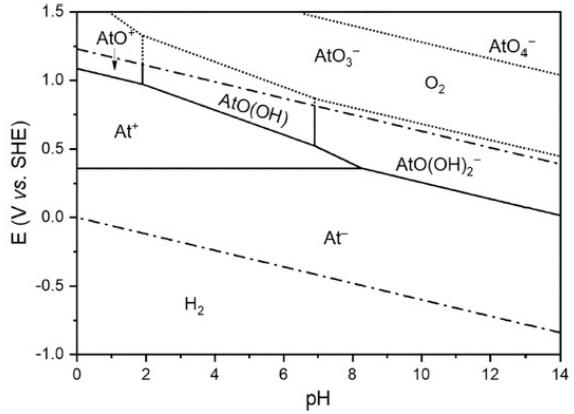


0%	0%	100%	0%	isolated state
75%	25%	0%	0%	+ solvation effects
9%	91%	0%	0%	+ SOC effects

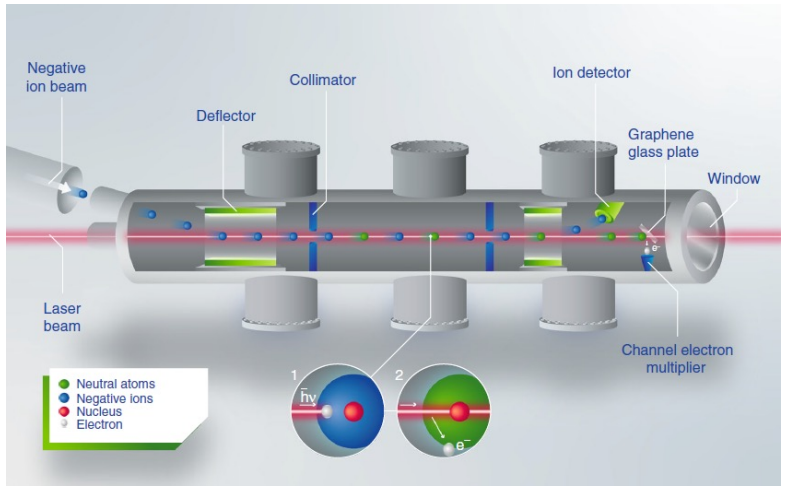
sophistication degree

Champion et al, Phys. Chem. Chem. Phys. 2011, 13, 14984

Astatine also behaves as a halogen



- Astatide (At^-) is stable in water
- Electron affinity reported in 2020. EA value in line with halogen trend



Experimental set up at CERN for EA determination by laser-photodetachment spectroscopy

EA = 2.41578(7) eV

I = 3,06 eV
Br = 3,36 eV
Cl = 3,62 eV
F = 3,40 eV

Table 1. Astatine's Atomic Properties Derived from the High-Precision Measurements of EA and IE, Compared to Those of Iodine[a]

Property	Definition	At	I
electronegativity	$\chi = \frac{IE + EA}{2}$	5.87 eV	6.76 eV
hardness	$\eta = \frac{IE - EA}{2}$	3.45 eV	3.70 eV
softness	$S = \frac{1}{2\eta}$	0.145 eV^{-1}	0.135 eV^{-1}
electrophilicity	$\omega = \frac{\chi^2}{2\eta}$	4.99 eV	6.17 eV

Leimbach et al. Nat Comm. 2020, *11*, 3824

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Astatine also behaves as a halogen

- Astatine forms halogen bonds

nature chemistry
ARTICLES
https://doi.org/10.1038/s41557-018-0011-1

Experimental and computational evidence of halogen bonds involving astatine

Ning Guo[1], Rémi Maurice[1], David Teze[1], Jérôme Graton[2], Julie Champion[1], Gilles Montavon[1]* and Nicolas Galland[2]*

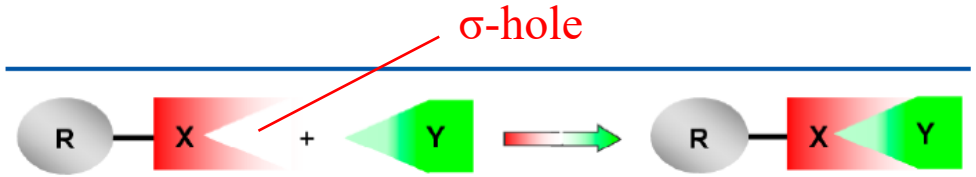
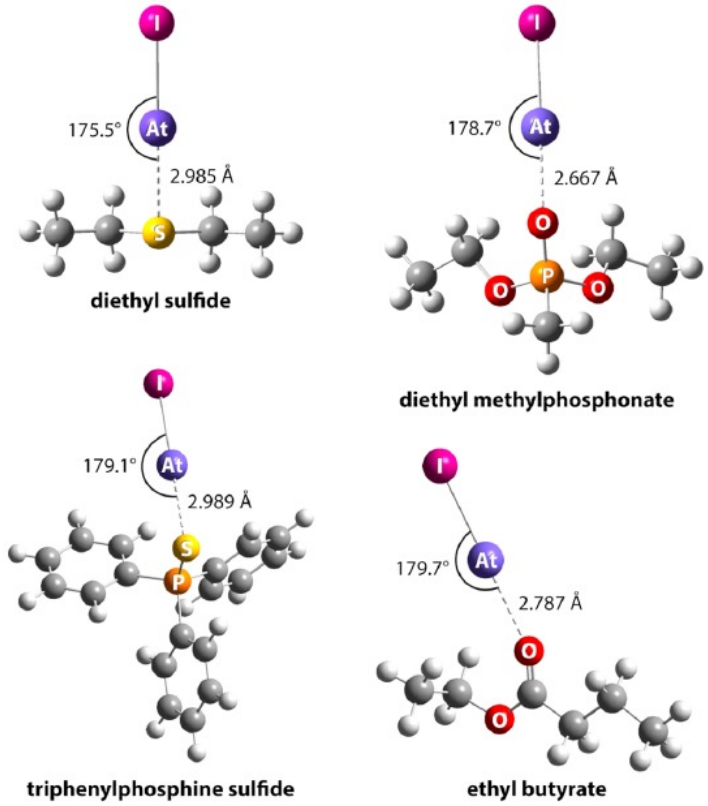


Figure 1. Schematic representation of the halogen bond.

At forms stronger X-bonds than iodine



Guo et al. Nat. Chem. 2018, *10*, 428

2- Improving labelling chemistry

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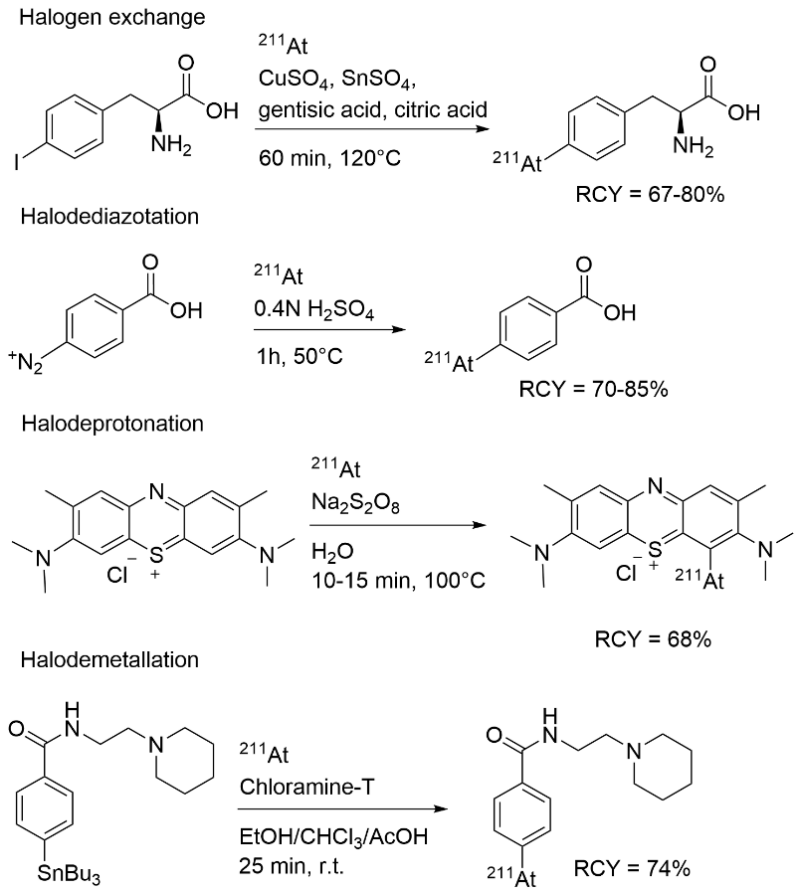
Radiolabelling Chemistry with Astatine-211: Improving Methods and In Vivo Stability

- Astatine labelling chemistry in the 1990-2000's

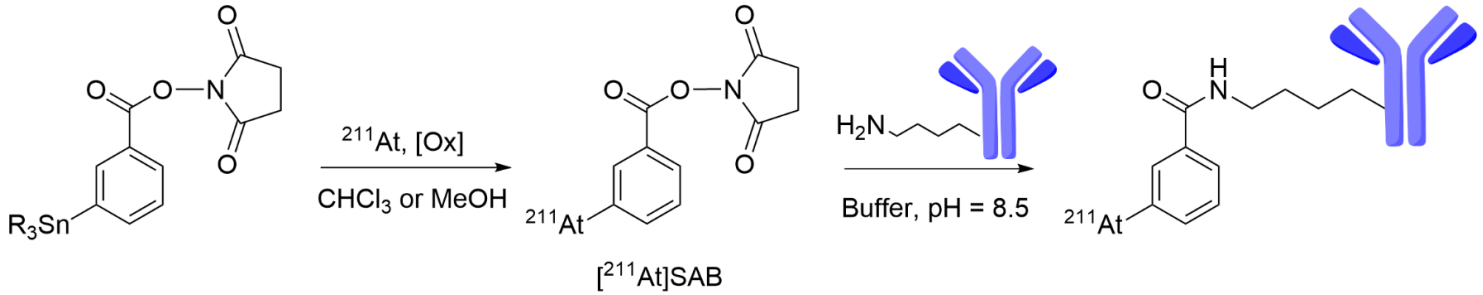


Astatine used as a typical halogen in radiolabelling reaction using nucleophilic (At^-) or electrophilic (At^+) species …

Guérard et al. Acc. Chem. Res. 2021, *54*, 3264-3275

Radiolabelling Chemistry with Astatine-211: Improving Methods and In Vivo Stability

- Astatine labeling chemistry in the 1990's-2000's



- lack of robustness of At^+ species
- purification issues

- suboptimal conjugation yields (50-75%)

- Gold standard for ≈ 30 years
- Used in the first 2 clinical trials (Zalutsky 2008 and Andersson 2009)
- Used in our first labelling studies, perfectible results

Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 12 June 2008, Revised 18 August 2008 Accepted 19 August 2008 Published online 24 September 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1543

Feasibility of the radioastatination of a monoclonal antibody with astatine-211 purified by wet extraction

Mickaël Bourgeois,[a,b]* François Guerard,[a] Cyrille Alliot,[a] Marie Mougin-Degraef,[a] Holisoa Rajérison,[a] Patricia Remaud-Le Saëc,[a] Jean-François Gestin,[a] François Davodeau,[a] Michel Chérel,[a] Jacques Barbet,[a] and Alain Faivre-Chauvet[a,b]

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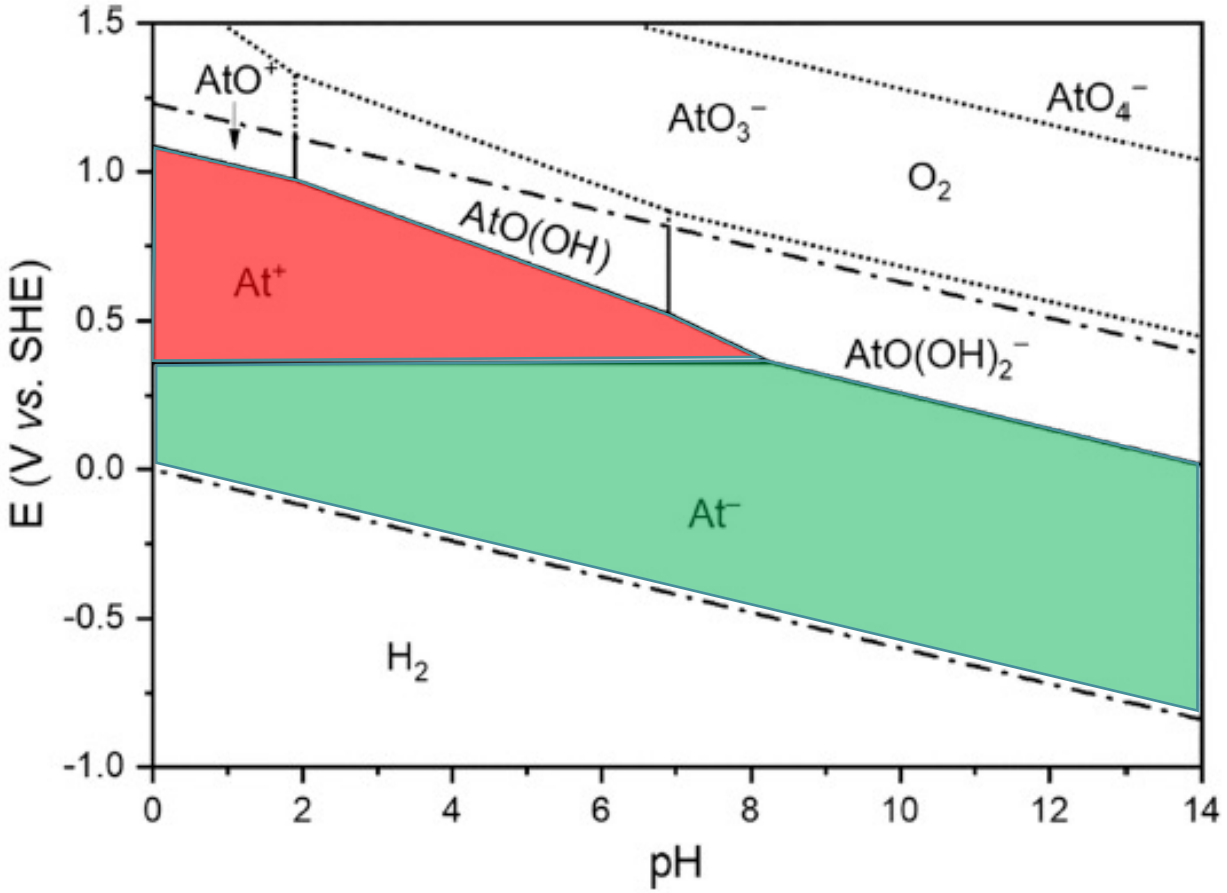
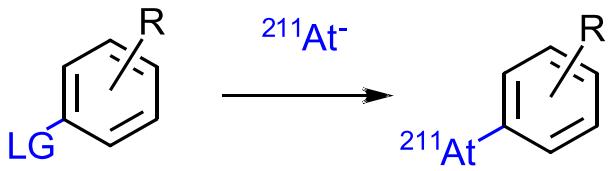
Development of nucleophilic ^{211}At labelling procedures

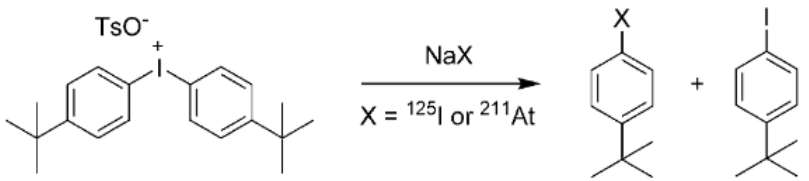




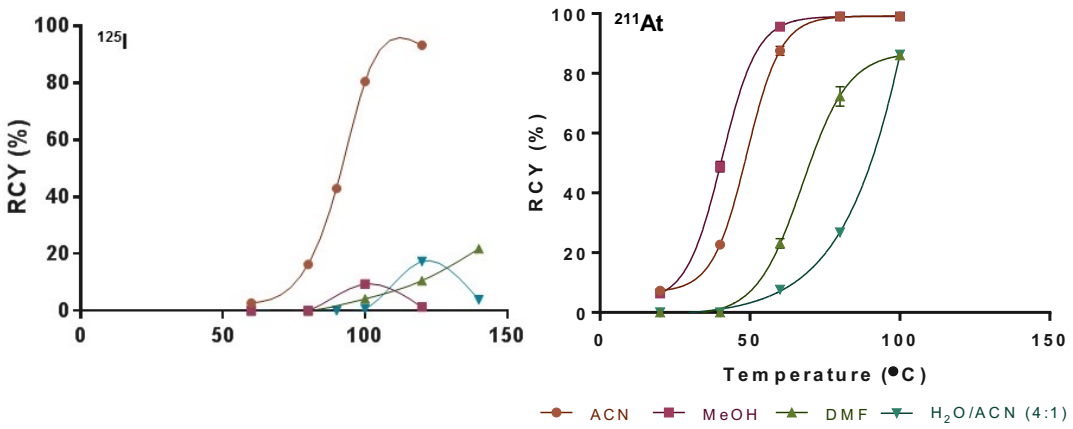
Which leaving group (LG) ?

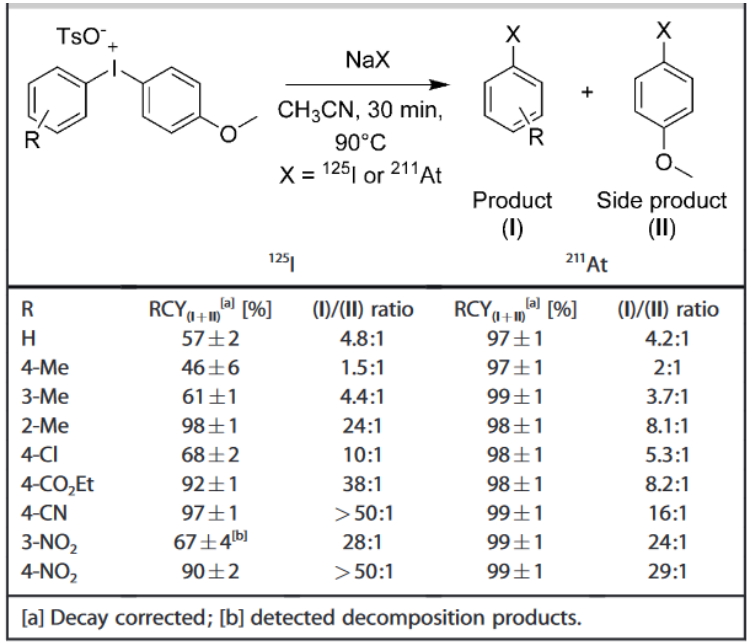
Development of nucleophilic ^{211}At labelling procedures

- Aryliodonium salts



At⁻ significantly more reactive than I⁻





R	$RCY_{(I+II)}$[a] [%]	(I)/(II) ratio	$RCY_{(I+II)}$[a] [%]	(I)/(II) ratio
H	57±2	4.8:1	97±1	4.2:1
4-Me	46±6	1.5:1	97±1	2:1
3-Me	61±1	4.4:1	99±1	3.7:1
2-Me	98±1	24:1	98±1	8.1:1
4-Cl	68±2	10:1	98±1	5.3:1
4-CO_2Et	92±1	38:1	98±1	8.2:1
4-CN	97±1	>50:1	99±1	16:1
3-NO_2	67±4[b]	28:1	99±1	24:1
4-NO_2	90±2	>50:1	99±1	29:1

[a] Decay corrected; [b] detected decomposition products.

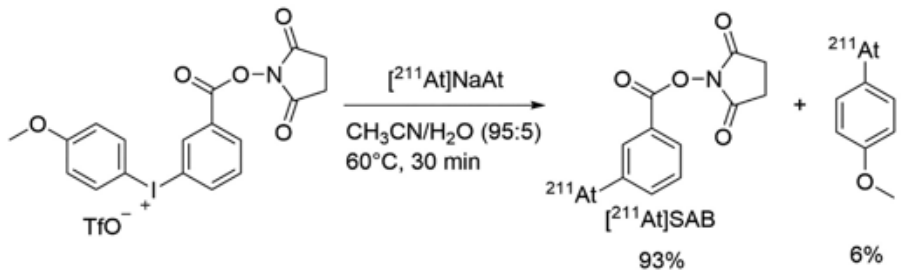
Increasing activating effect

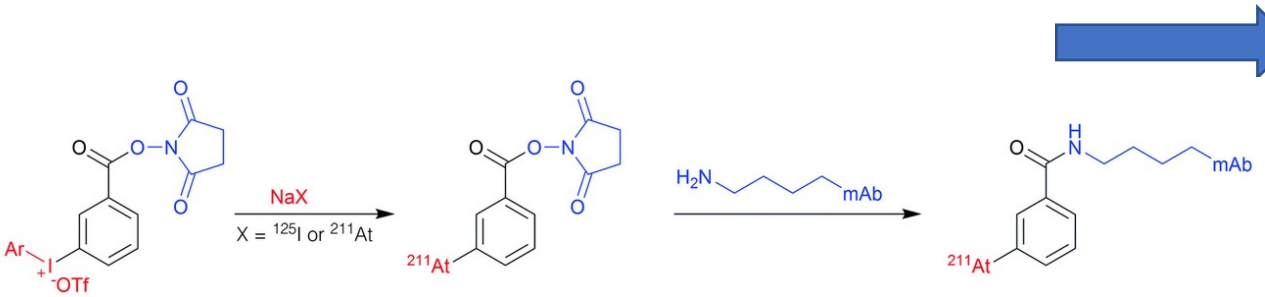
Increasing (I)/(II) ratio

Guérard et al, Chem. Eur. J. 2016, 22, 12332-12339

Development of nucleophilic ^{211}At labelling procedures

- Aryliodonium salts: application to mAb ^{211}At-labeling





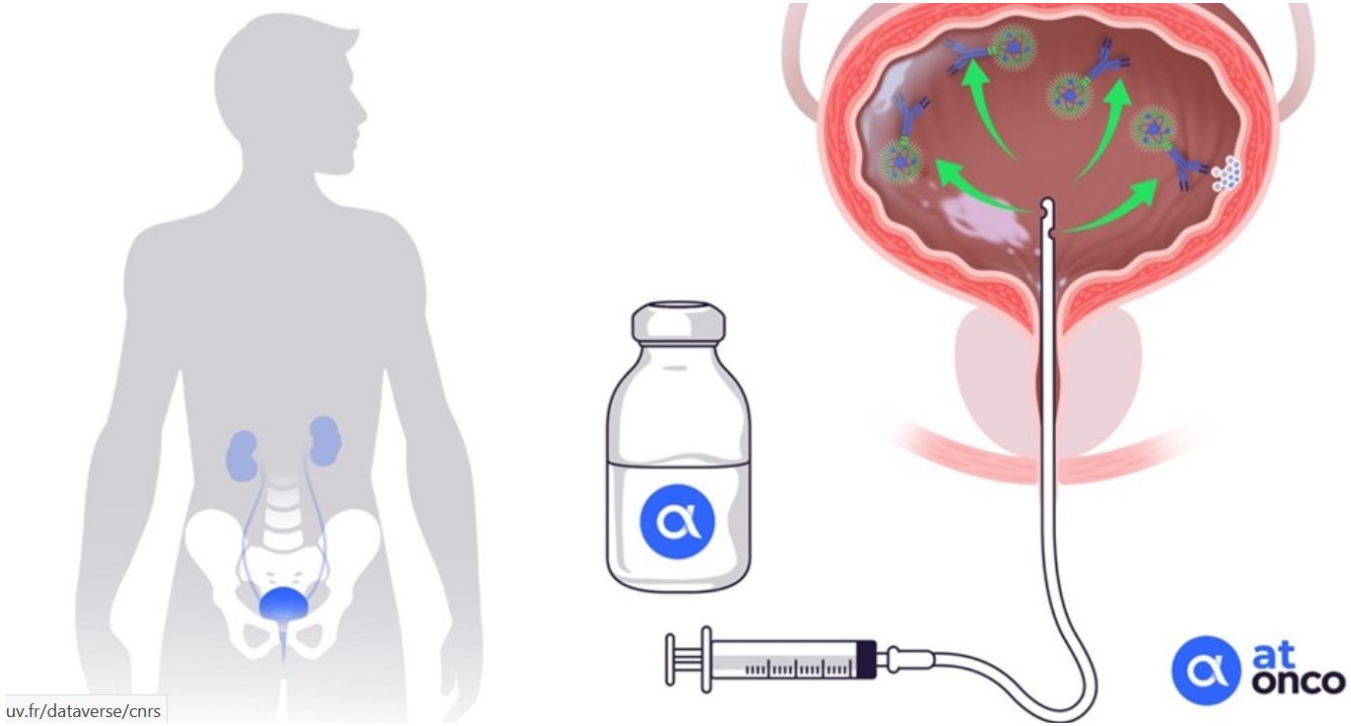
Highly robust procedure adopted for routine production of ^{211}At-labeled mAb

15.15	Brain intratumoral At-211 radiotherapy targeting syndecan-1 leads to durable glioblastoma remission and immune memory in female mice	Michel Chérel – CRCI²NA lab, Nantes Université
15.35	Advanced image analysis in nuclear medicine: an illustration from Nantes	Thomas Carlier – University Hospital of Nantes
15.55	Coffee break	
16.10	PET imaging and targeted alpha therapy in a multiple myeloma mouse model	Sébastien Gouard – CRCI²NA lab, Nantes Université
16.30	Review on the XEMIS2 camera installation	Nicolas Beaupère – Subatech

Guérard et al, Bioorg. Med. Chem. 2017, *25*, 5975-5985

Development of nucleophilic ^{211}At labelling procedures

- Aryliodonium salts: application to mAb ^{211}At-labeling



Clinical trial in preparation for bladder cancer treatment with Atonco start up

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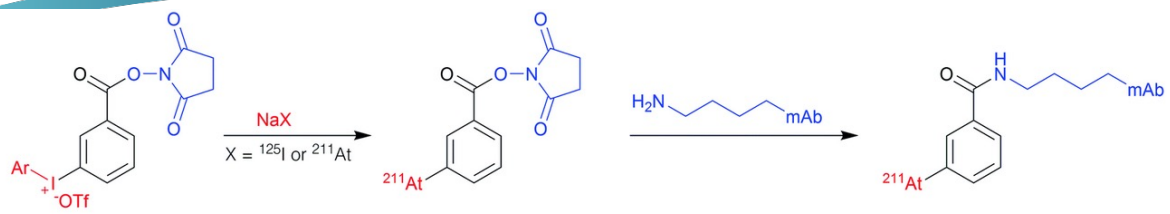
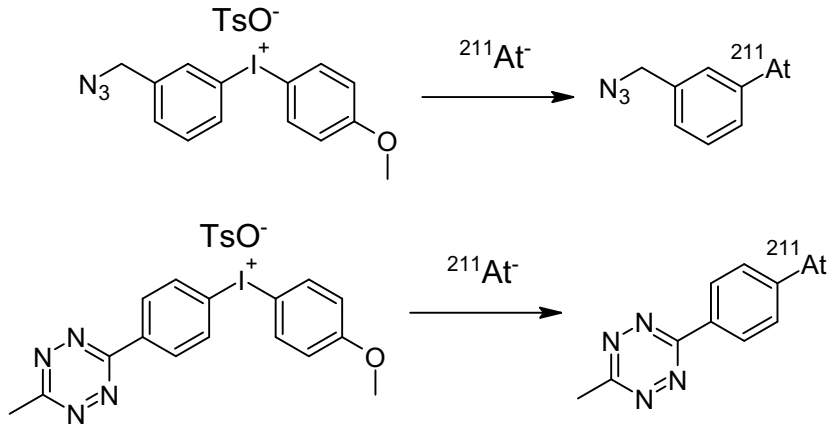
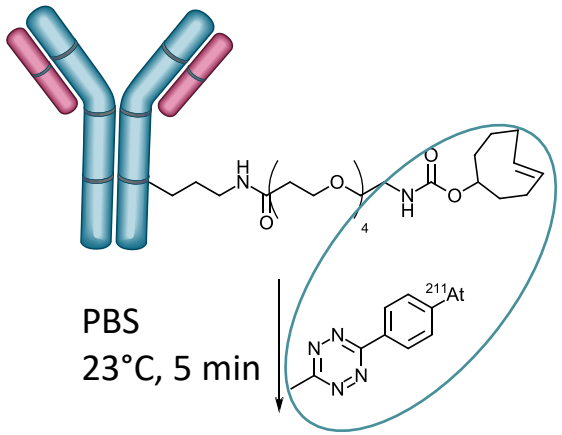
Click chemistry to improve bioconjugation step



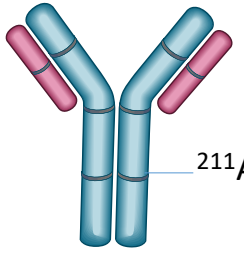
Conjugation yield is only 50-60%



TCO/Tz-IEDDA



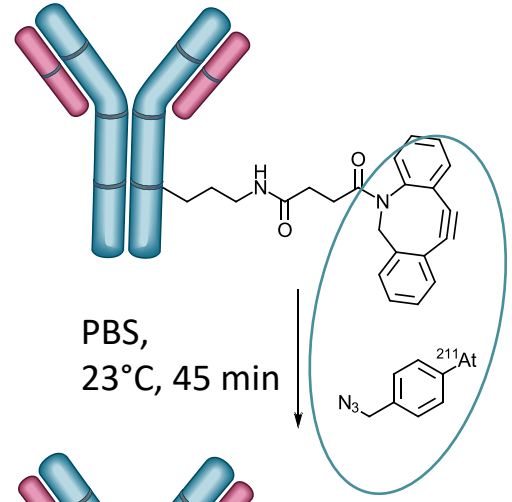
PBS
23°C, 5 min



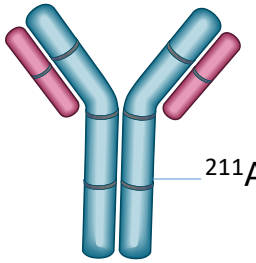
RRC = 92 ± 4%

Immunoreactivité : 82 ± 3%

DIBAC/N_3-SPAAC



PBS,
23°C, 45 min



RRC = 90%

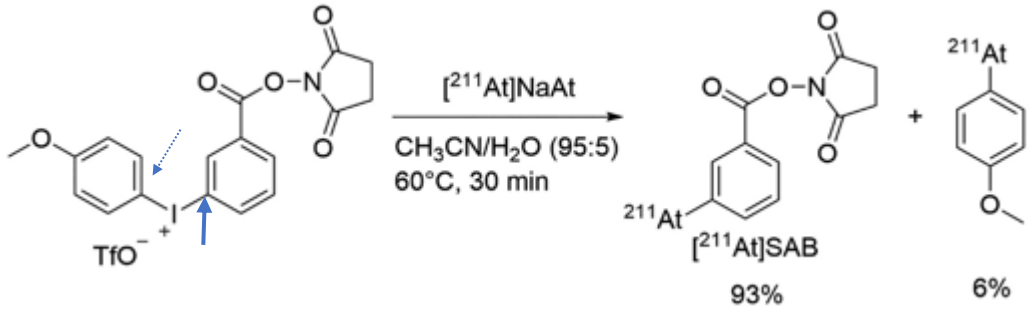
Immunoreactivité: 82 ± 5%

Navarro, Bioorg. Med. Chem. 2019, 27, 167

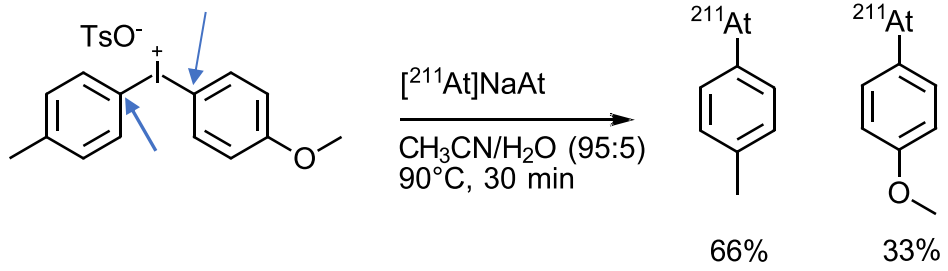
Development of nucleophilic ^{211}At labelling procedures

- Aryliodonium **ylides** for improved regioselectivity and RCYs

Aryliodonium **salts**

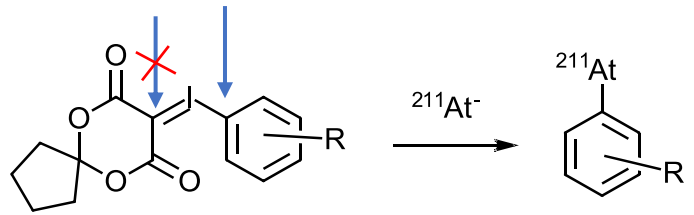


Good selectivity with electron deficient aryl



Low selectivity with electron rich aryl

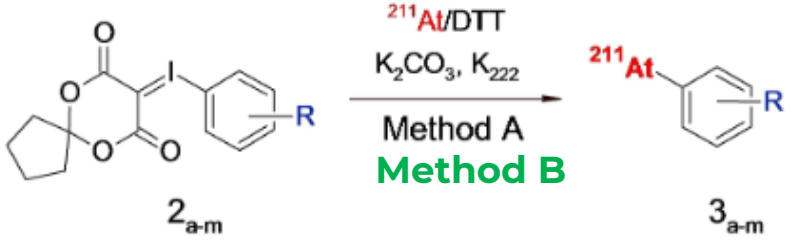
Aryliodonium **ylides**



1 possible product expected

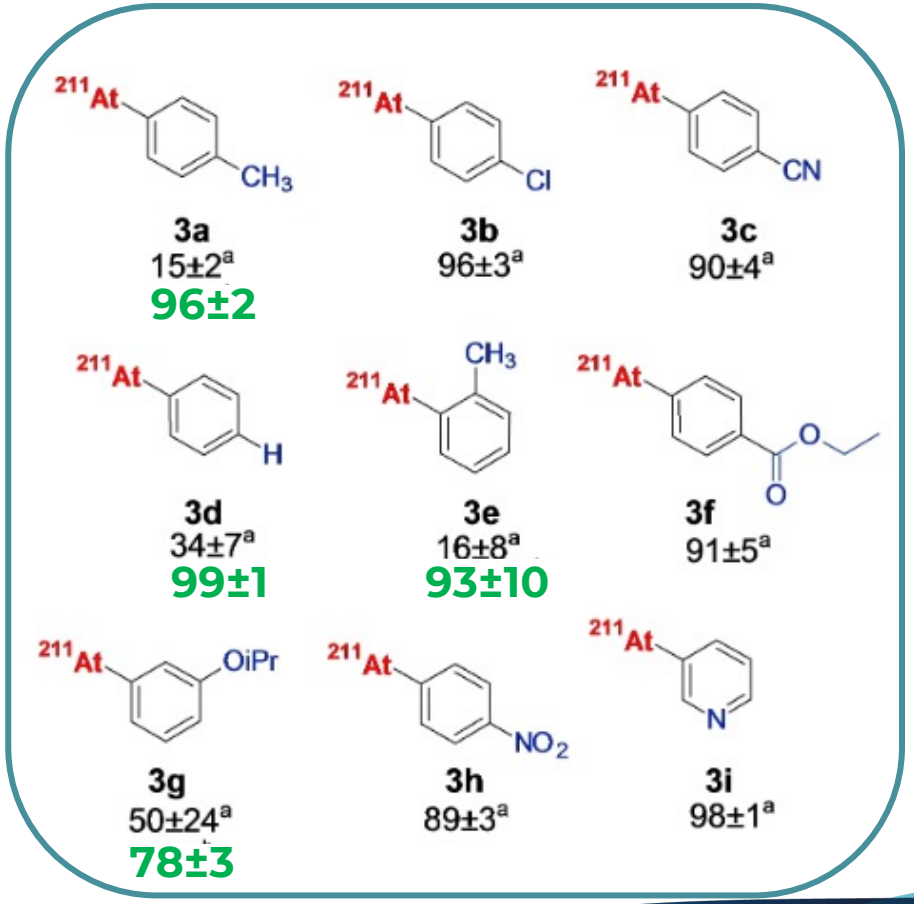
Development of nucleophilic ^{211}At labelling procedures

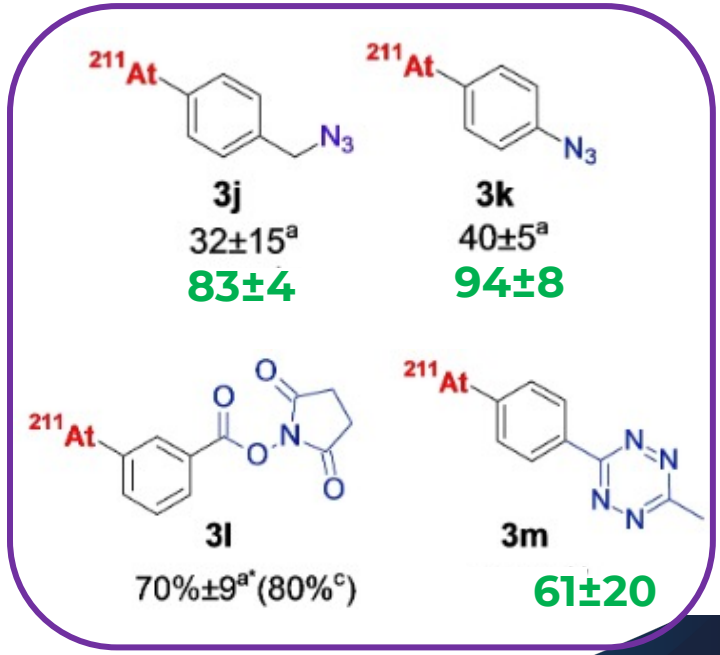
- Aryliodonium ylides for improved regioselectivity and RCYs



Method A: CH_3CN, 20°C, 30 min (activated compounds)

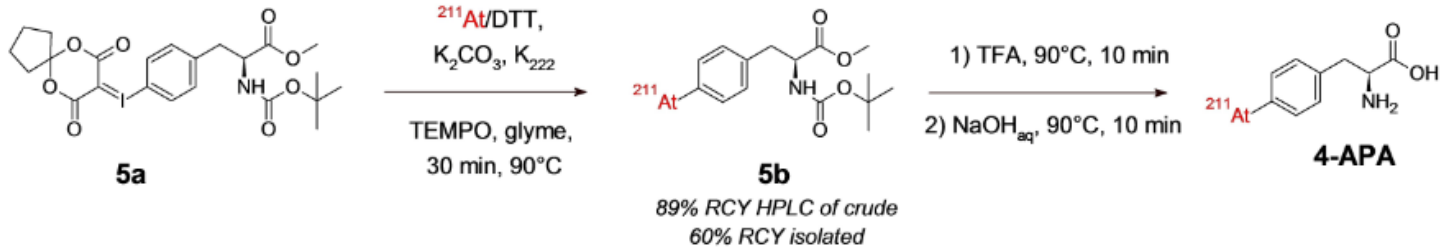
Method B: Glyme, TEMPO, 90°C, 30 min (deactivated compounds)





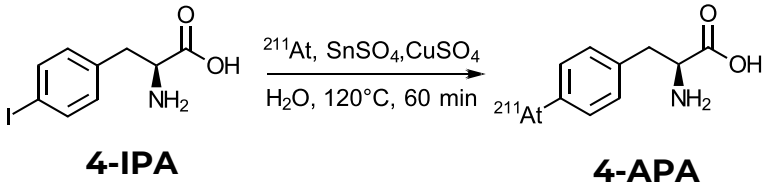
Development of nucleophilic ^{211}At labelling procedures

- Aryliodonium ylides for improved regioselectivity and RCYs

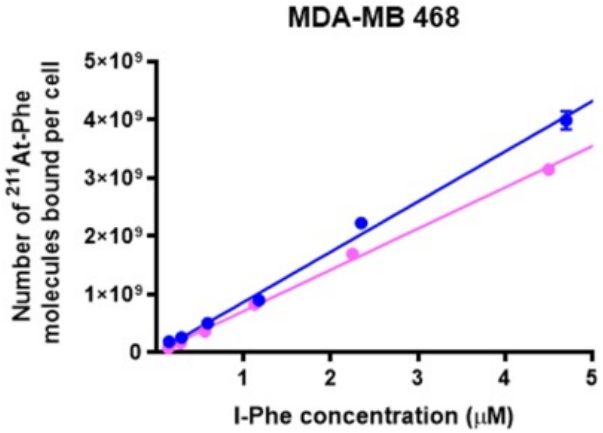


AY = 27 MBq
Am = 620 MBq/µmol
(starting from 70MBq)

Classical halogen exchange



AY = 27 MBq
Am = 9.5 MBq/µmol
(starting from 77MBq)



- Iodonium ylide
- classical

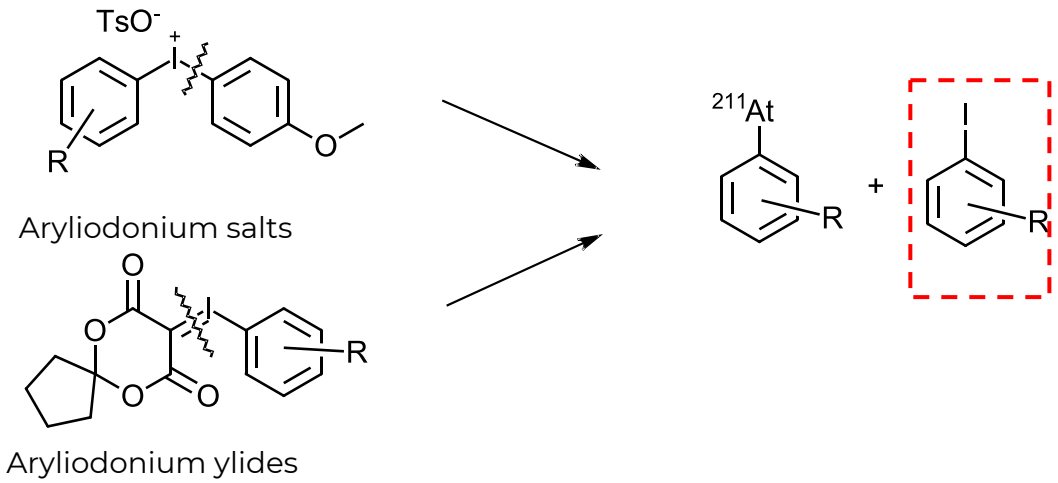
Preclinical therapy studies of multiple myeloma 4-APA designed Orphan drug by FDA in 2020.

No impact of method on cell uptake

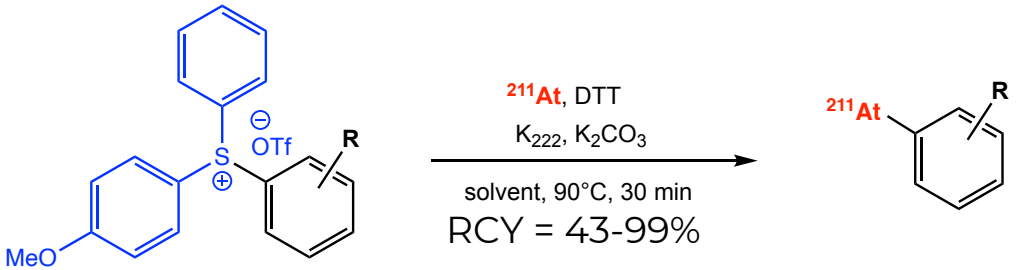
Maingueneau et al, Chem. Eur. J. 2022, *28*, e202104169

Development of nucleophilic ^{211}At labelling procedures

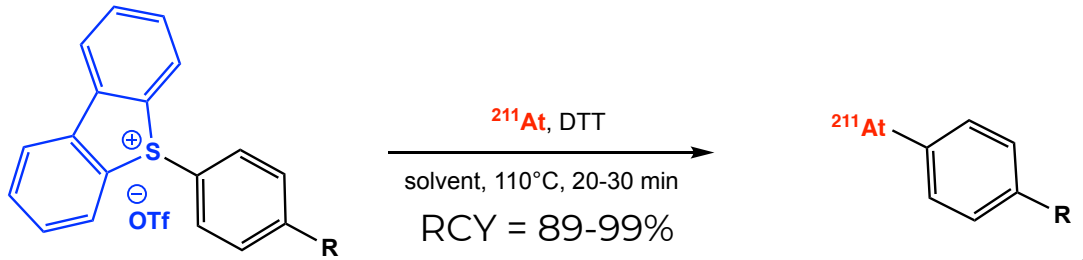
- **Arylsulfonium salts** for improved molar activity



Arylsulfonium salts



R = H, p-Me, o-Me p-Cl, p-NO_2



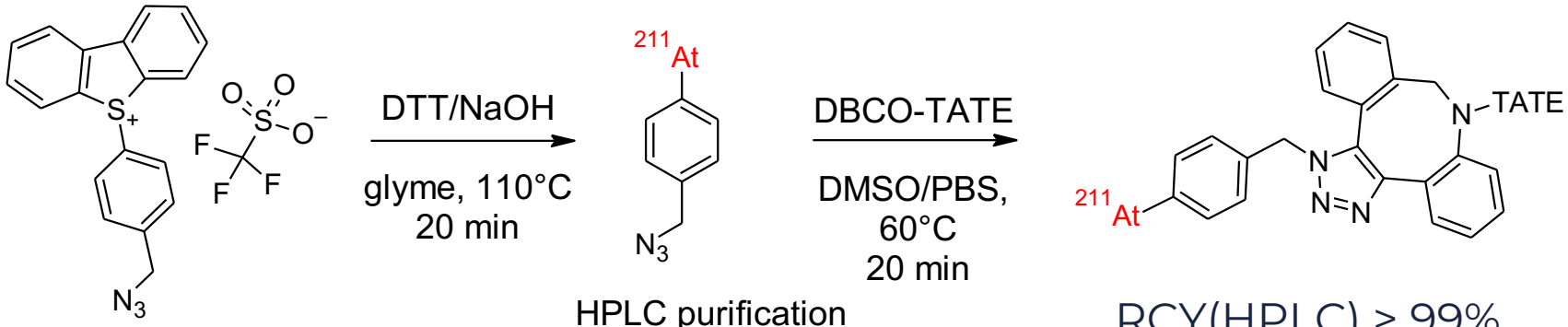
R = 4-Me, 4-Cl, 4-CN, 4-NO_2

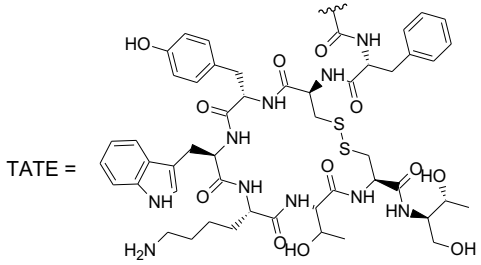
Inseparable iodinated decomposition product
=> Limits the molar activity (Bq/mol) achievable

Maingueneau et al, Nucl. Med. Biol. 2022,108-109, S43-S44

Development of nucleophilic ^{211}At labelling procedures

- **Arylsulfonium salts** application to peptide labelling





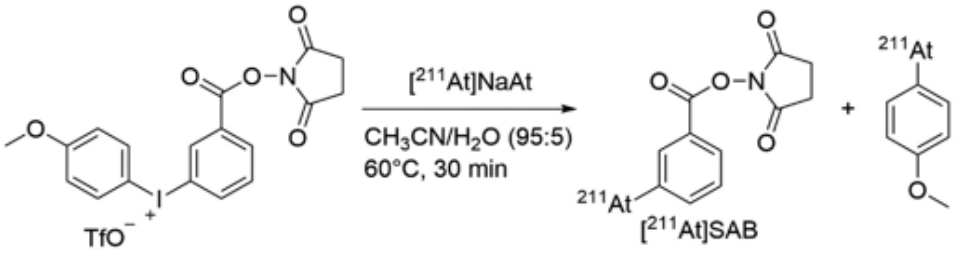
RCY(HPLC) > 99%
Starting with 20µg peptide

Maingueneau et al, Nucl. Med. Biol. 2022,108-109, S43-S44

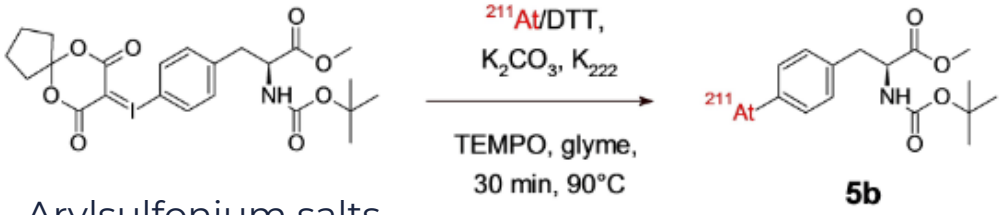
Development of nucleophilic ^{211}At labelling procedures

- 1-step labelling of proteins?

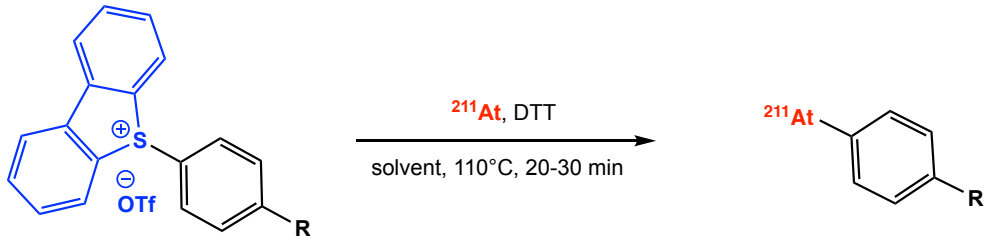
Aryliodonium salts

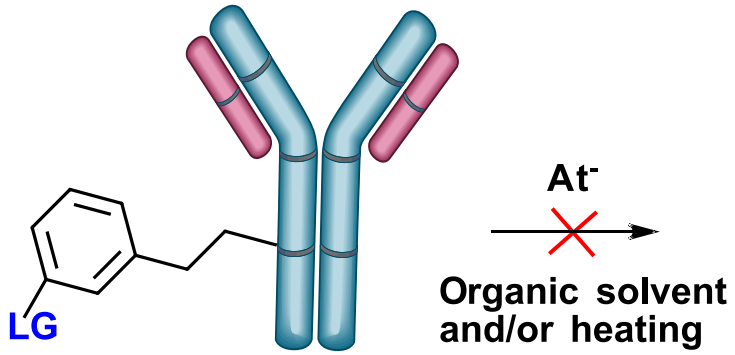


Aryliodonium ylides



Arylsulfonium salts



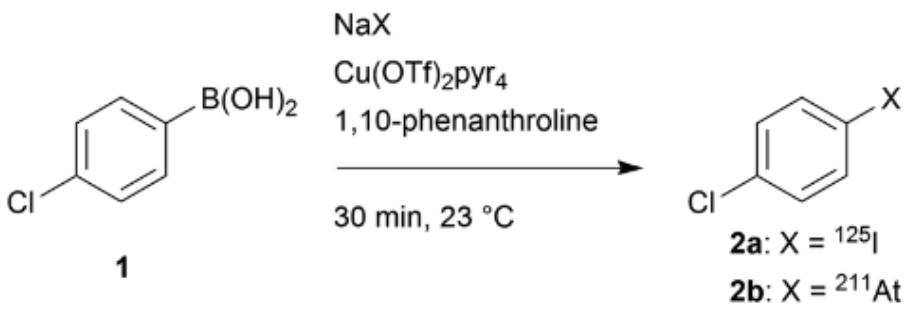


Protein denaturation

Is there a LG that would be compatible for radiolabelling at low temperature in water?

Development of nucleophilic ^{211}At labelling procedures

- Arylboronic acids for 1-step labelling of proteins

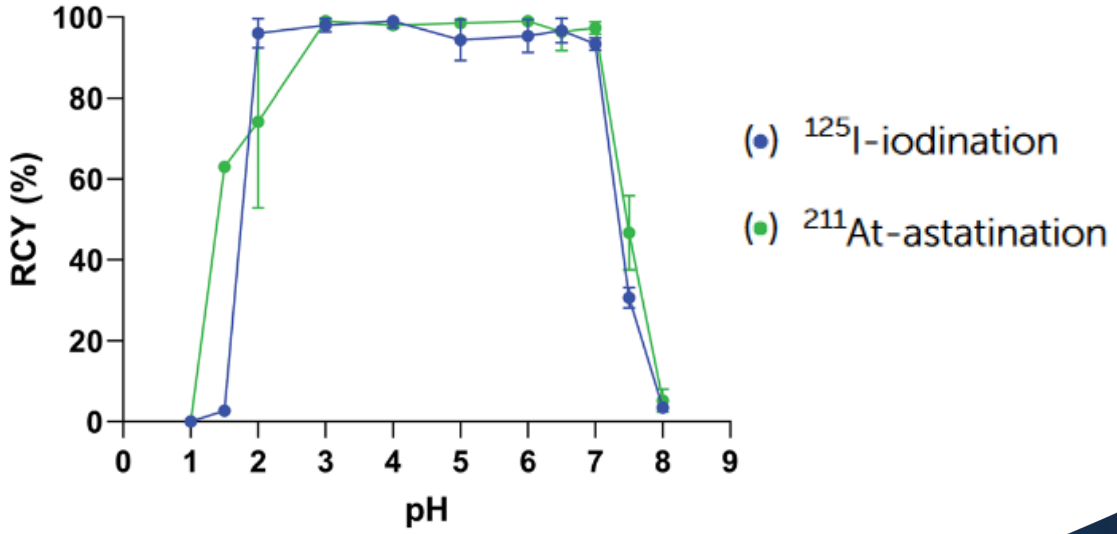


1

2a: X = ^{125}I

2b: X = ^{211}At

Solvent = MeOH or H_2O (+15% DMSO): RCY > 99%



Large pH window application

Berdal et al, Chem. Sci. 2021, *12*, 1458

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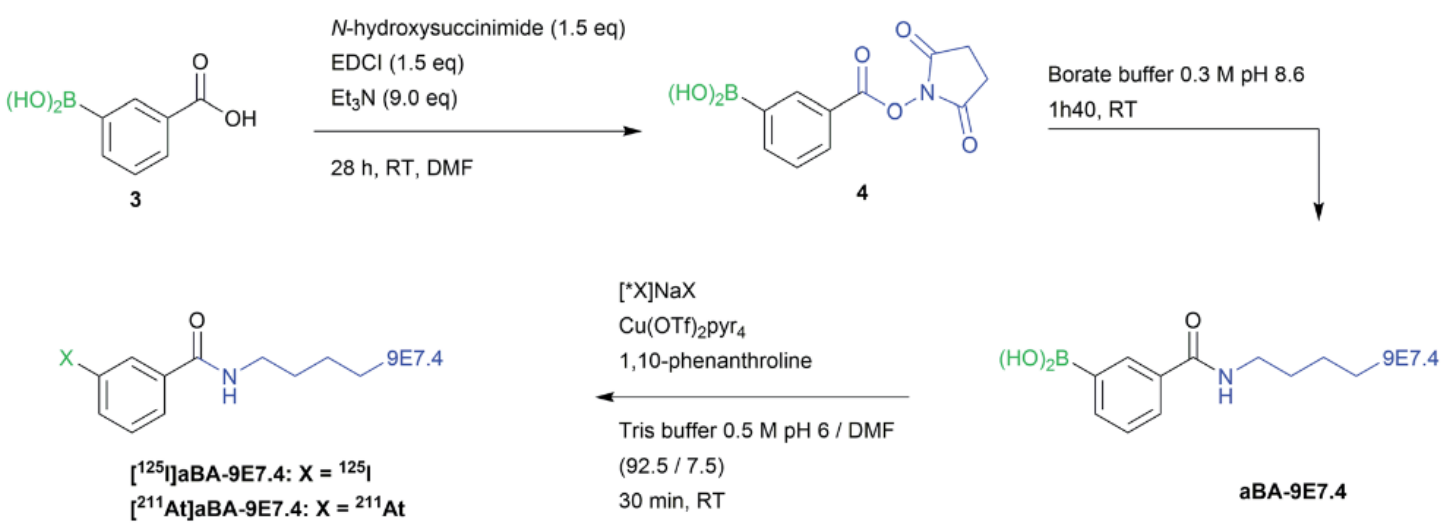
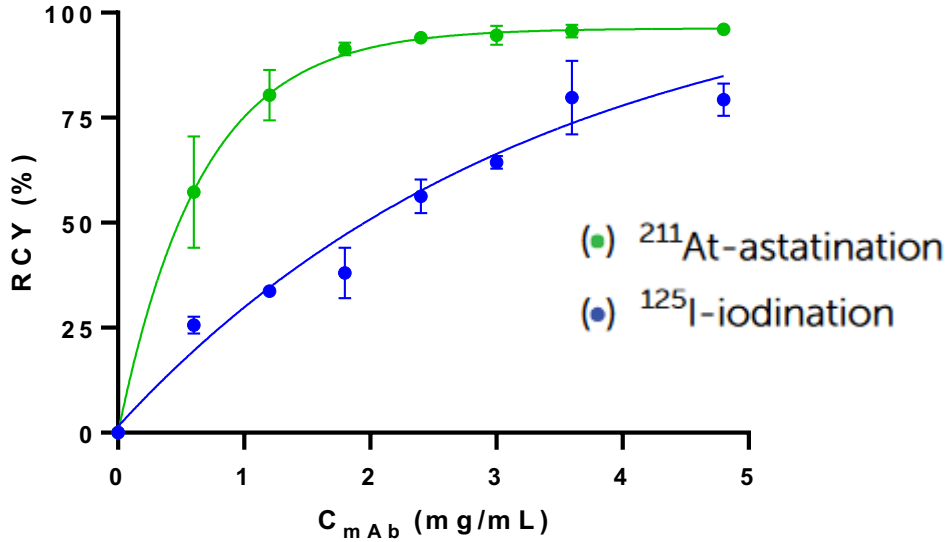
Development of nucleophilic ^{211}At labelling procedures

- Arylboronic acids for 1-step labelling of proteins





- ✓ Improved RCY and specific activity (MBq/mg) compared to 2-step procedures
- ✓ Unchanged biodistribution compared to 2-step procedures
- ✓ Preconjugated mAb storable in labelling buffer > 1 year without decrease in RCY

Berdal et al, Chem. Sci. 2021, *12*, 1458

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3-Understanding the stability issue

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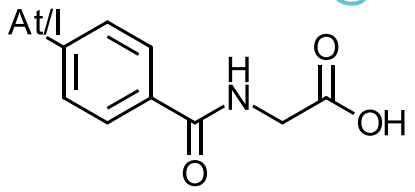
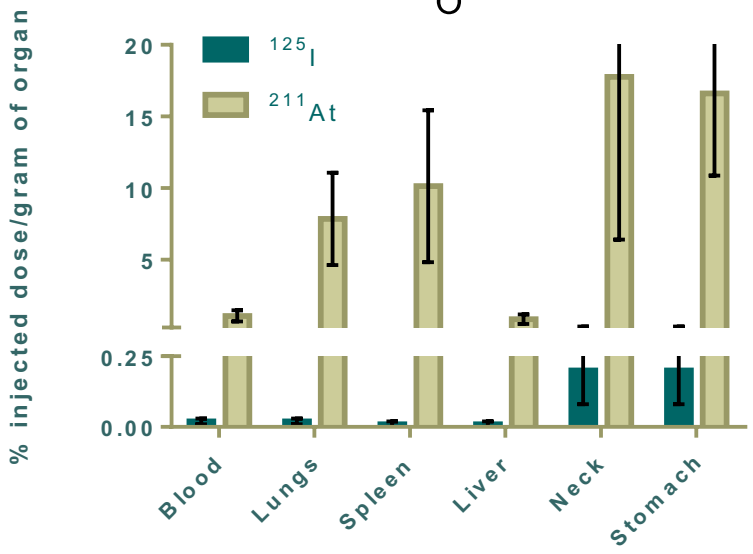
Understanding and improving in vivo stability of ^{211}At-labelling

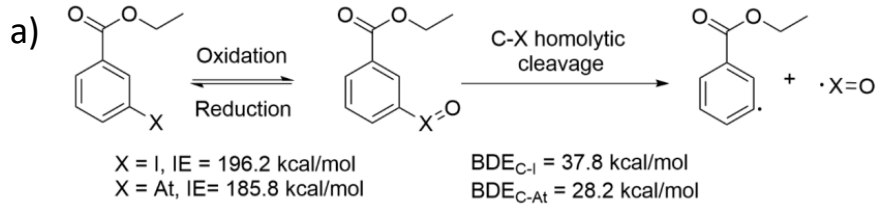
- At-labelled compounds significantly less stable in vivo than radioiodinated analogues

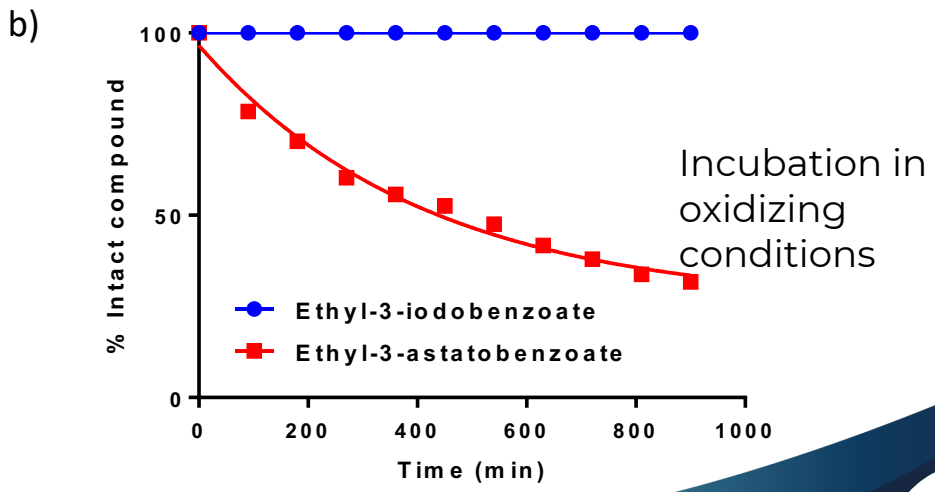




Para-halogenohyppuric acids 4 h after injection in mice

Oxidative dehalogenation ?



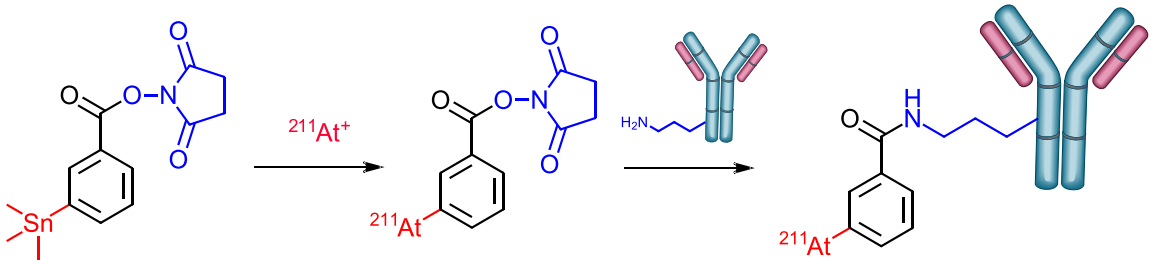


Incubation in oxidizing conditions

Teze et al, Sci. Rep. 2017, 7, 2579

Understanding and improving in vivo stability of ^{211}At-labelling

- Selenium mediated deastatination

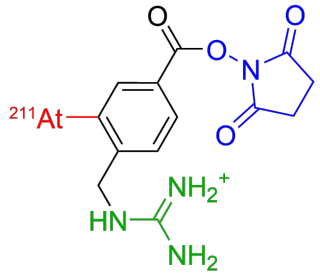


SAB

SAB = suboptimal stability

12.00	Is astatine the next workhorse of targeted alphatherapy? Pluridisciplinary perspectives	François Guerard – CRCI²NA lab, Nantes Université
12.30	How chemical simulations can help driving new radiolabelling strategy?	Samuel Mador – CEISAM lab, Nantes Université
13.00	Lunch break	

SAGMB as alternative prosthetic group



SAGMB = improved stability

guanidinium group

Yssartier, RSC Med. Chem. 2024, 15, 223

Conclusions

- **Astatine-211 exhibits excellent characteristics for targeted α therapy**
- **Availability has long been limited but is now rapidly increasing**
- **Chemistry is challenging due to limited knowledge in basic properties of At and is the object of new studies**
- **Radiolabelling chemistry is now enriching fast, with more efficient and more robust methods becoming available**
- **Stability issue remains to be better understood and resolved**

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Future directions

- **Keep improving efficiency of labelling procedure**
- **Application to a broader scope of targets (from small molecules to proteins)**
- **Clinical use**
- **Stability:**
  - **improving the understanding of deastatination mecanisms to propose solutions**
  - **Investigating new bonding modalities (ERC SAt-Radio)**

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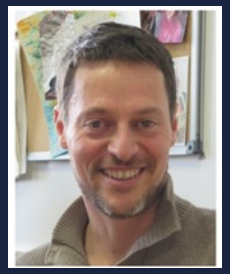
Acknowledgement

Nuclear Oncology Team
(Michel Chérel & Françoise Kraeber-Bodéré)

Nuclear Oncology Team Nantes

Arronax Nantes

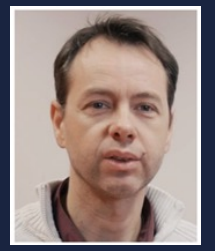
Radioanalytical chemistry



Subatech

Dr. Gilles Montavon

Molecular Modeling



CEISAM
Chimie et Interdisciplinarité Synthèse, Analyse, Modélisation

Dr. Nicolas Galland

iron labex

SIRIC ILIAD
Nantes - Angers

NOAR
NETWORK FOR OPTIMIZED ASTATINE LABELED RADIOPHARMACEUTICALS

European Research Council
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