FRAGMENT SCREENING AT THE SWEDISH NMR CENTRE

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SWEDISH NMR CENTRE

- Provides national NMR support
- Located at GU and UmU
 - Material Science

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- Structural biology
- Biogeochemistry
- Environmental chemistry
- Translational NMR
 - Metabolomics
 - Small molecule analysis
 - FBS



Publication by Journal Impact Factor





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AFFINITY DETECTION RANGES FOR NMR

NMR screening experiment very well suited for detection of weak binders!



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Ligand-observed experiments



- Common primary screening method
- No size limitations of target
- No isotope labelling required
- No information about binding site
- "Low" protein concentration, low μM

Protein-observed experiments



- Detects binding and also binding site
- Follow-up of hits
- Requires isotope labelling (15N/13C)
- Size limit of protein (<50kDa)
- Higher protein concentration (>100 μ M)

FRAGMENT LIBRARIES

- Maybridge
 - 800 fragments
 - 1000 originally
 - 100mM stock solutions (DMSO-d6)
- Bionet 19F
 - 428 19F-labelled fragments
 - 462 originally
 - 25mM stock solutions (DMSO-d6)
 - Acquired by CBCS together with Technical University of Denmark (DTU)
- User supplied libraries can also be used





QUALITY CONTROL OF FRAGMENT LIBRARIES

- Can we trust what we're buying?
 - Correct structure?
 - Solubility?
 - Purity?
 - http://practicalfragments.blogspot.com/201 4/10/caveat-emptor.html
 - 0-33% failure rate, 16% average
- Shelf-life?
 - Time
 - Storage

• NMR reference spectra recorded under "screen-like" conditions

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- 80

- 75

• Maybridge

- 65

- 1000 cmpds
- 800 passed QC
- Bionet

- 60

- 461 cmpds
- 428 passed QC

- 70

δ 19F (ppm)

¹⁹F FRAGMENTS

- Why do we use fluorinated fragments?
- Pros
 - Almost same sensitivity as for ¹H
 - Single peak for each fragment (mostly)
 - Low risk of overlapping peaks
 - More pronounced binding response
 - Allows for additives in the samples
 - Glycerol, detergents etc.
 - No need for deuterated solvents
- Cons
 - Limited availability of fluorinated cmpds
 - Wide spectral width (can be overcome)



SCREENING PROCEDURE

- 100μM fragment, 5-10μM protein
- 10 fragments in each sample
- Bionet 19F library
 - T2-experiment
 - Approx 30h for screening (84 samples, 20 min per sample)
 - ~3mg protein (30kDa)
 - Cost approx 10 000 SEK (academic users)
- Maybridge 1H library
 - T1rho/WLOGSY/STD experiments
 - Approx 4 days for screening (160 samples, 40 min per samples)
 - ~5mg protein (30kDa)
 - Cost approx 15 000 SEK (academic users)

	V(frag)	Library	Library plates	
			2	6
	Deep-well plate	3	4	
		5	6	
		7	8	
		9	10	
Blank				Ĵ
Protein				2

EQUIPMENT

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600 MHz (UmU)



700 MHz (GU)



SamplePro pipetting robot (UmU/GU)

FBS-EXAMPLE

Bcl-2, mitochondrial membrane protein involved in apoptosis

- Detergent (DPC) needed for protein stability
- Reference spectra of all fragment mixes also recorded in ٠ presence of DPC (empty micelles)
- 19F screening (Bionet library) ٠
- 8 binders ($\sim 2\%$ hit rate) ٠
- 11 fragments without signal in reference spectra
 - Micelle binders ٠

Ref



Mushtag et al, Molecules, 2021, 26, 1467

PROJECT APPLICATIONS

- <u>https://www.gu.se/en/nmr/access-to-the-</u> <u>infrastructure</u>
- <u>www.swednmr.se</u> (under construction)
- Continous project evaluation
- First come, first serve

Applications for all types of NMR applications are welcome!









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