**Notes for Discussion: PAINS**

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* **What are PAINS?**
	+ **P**an-**A**ssay **IN**terference compound**S** (frequent hitters, false positives, non-specifics etc.)
	+ Use multiple, different sites on enzyme
	+ Widespread in biochemical HI enzyme activity assays
	+ Can be the test compound and/or its impurities
	+ Often not recognised as non-specific ⇨ Confusion, wasted time and money
* **Many Mechanisms**
	+ Technology interference
* Generate reactive species
	+ Reactive
* Redox
	+ Form molecular aggregates
	+ Insolubility
* Promote denaturation by binding unfolded protein
* **Identification Guide**
	+ IC50 > 3µM
	+ IC50 shift with [E]
	+ Steep concentration-inhibition curve
	(super-stoichiometric)
	+ Non-competitive
	+ Irreversible
	+ Time-dependent
	+ Active in unrelated screens
* Sensitive to detergent (aggregators)
* No comparable activity in orthogonal assay (assay with different physical basis)
* A known or predicted PAINS structure
* Flat SAR within similar compounds
* A PAINS is not necessarily a PAINS in all assays
* **Avoidance**
	+ Don’t remove from screening set
	+ Chelators
	+ Ensure enzyme is assay-stable
* Reductant
* Detergent
* Share new techniques and PAINS

* **Position Statement**
	+ Aim to identify and avoid PAINS in early drug discovery and publications
	+ Keep PAINS in screening sets and flagged (computational and historical data)
	+ Develop a robust PAINS assay cascade specific to target
	+ Determine and strengthen hit SAR early
	+ MOI only when hit structure and SAR demonstrated
	+ Share PAINS management best practice & learning
* **Discussion points**
	+ PAINS property guidelines - What’s the most important and why?
	+ Early HI screening – What makes an efficient screening cascade to avoid PAINS?