

1. Introduction - Sepsis

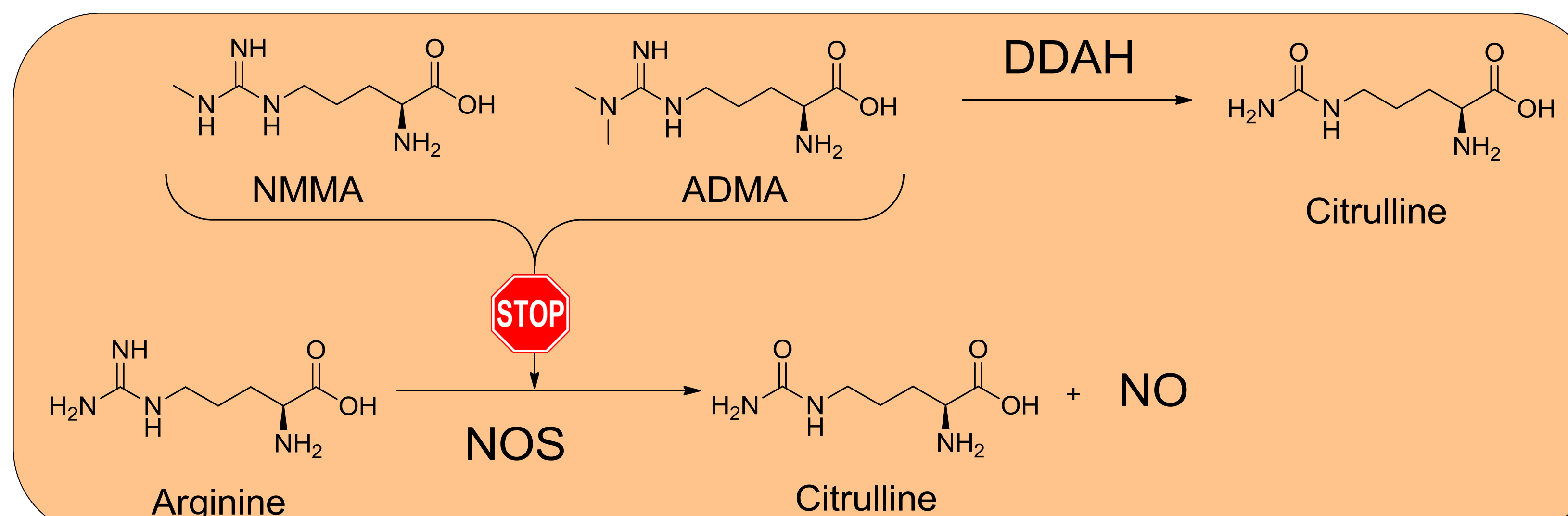
- The nitric oxide (NO) pathway has been implicated in numerous disease states, including cardiovascular disease, pain, inflammation, sepsis and cancer¹
- Acute sepsis constitutes a major threat to intensive care unit patients and its treatment is a large unmet clinical need
- Approximately 1 in 4 patients dies as a result of developing sepsis, which equates to hundreds of thousands of patient deaths worldwide, per year²
- Sepsis occurs when the body elicits an excessive inflammatory response to a microbial infection. This can lead to multiple downstream pathophysiological effects, including the production of iNOS (the inducible form of NO synthase) and excessive NO generation. This can ultimately lead to vascular collapse and death
- In an on-going programme, inhibitors of dimethylarginine dimethylaminohydrolase (DDAH) have been targeted for the treatment of vascular collapse during sepsis infection

References

1. Leiper, J. *et al. Nature Reviews Drug Discovery* 10, 277-291 (April 2011); 2. Angus, D. C. *et al. Crit. Care Med.* 29, 1303-1310 (2001). Karlsson, S. *et al. Intensive Care Med.* 33, 435-43 (2007). Blanco, J. *et al. Crit. Care* 12, R158 (2008); 3. Rossiter, S. *et al. J. Med. Chem.* 2005, 48, 4670-4678

2. DDAH1 as a target for Sepsis

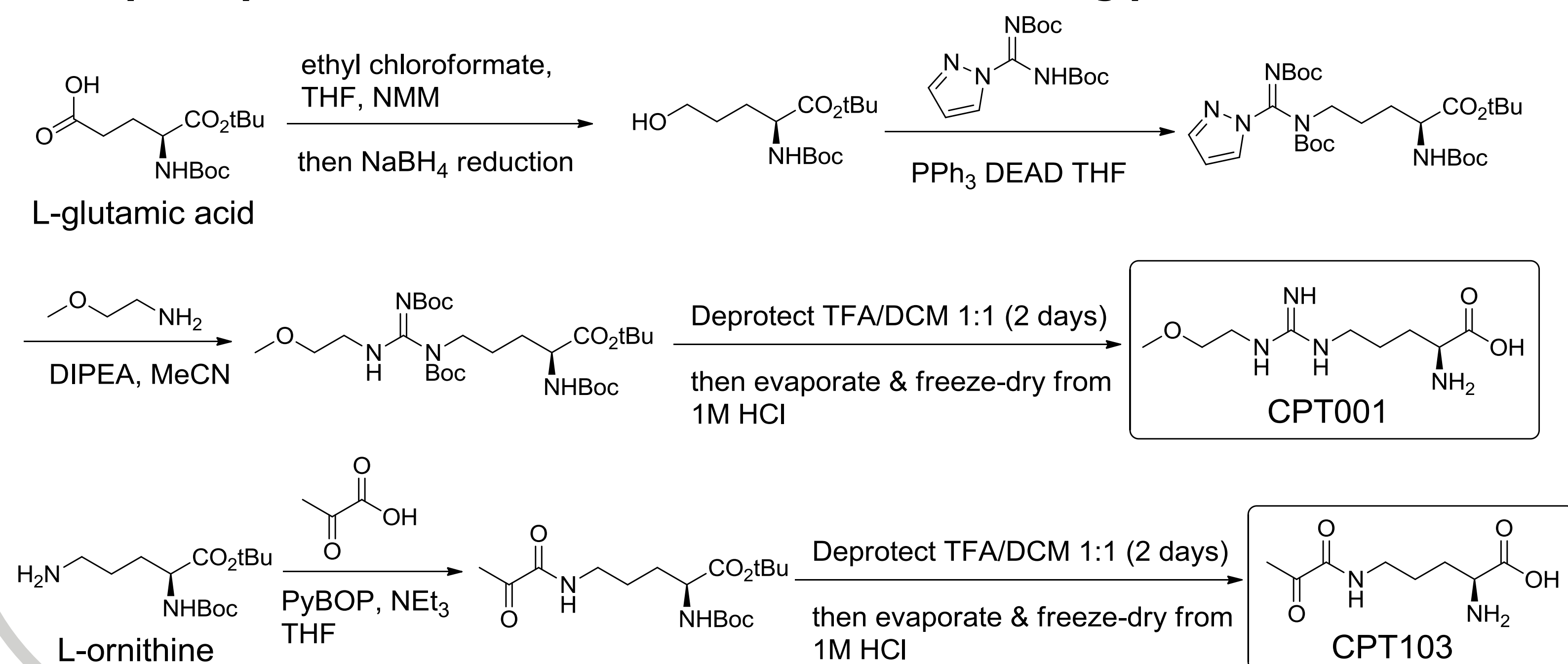
- DDAH metabolises two endogenous methylarginines, L-N-methylarginine (NMMA) and asymmetric L-N,N-dimethylarginine (ADMA), to give citrulline
- These endogenous substrates for DDAH are inhibitors of nitric oxide synthases (NOS); hence inhibition of DDAH indirectly inhibits NOS, and potentially affects those diseases where excess NO production is implicated



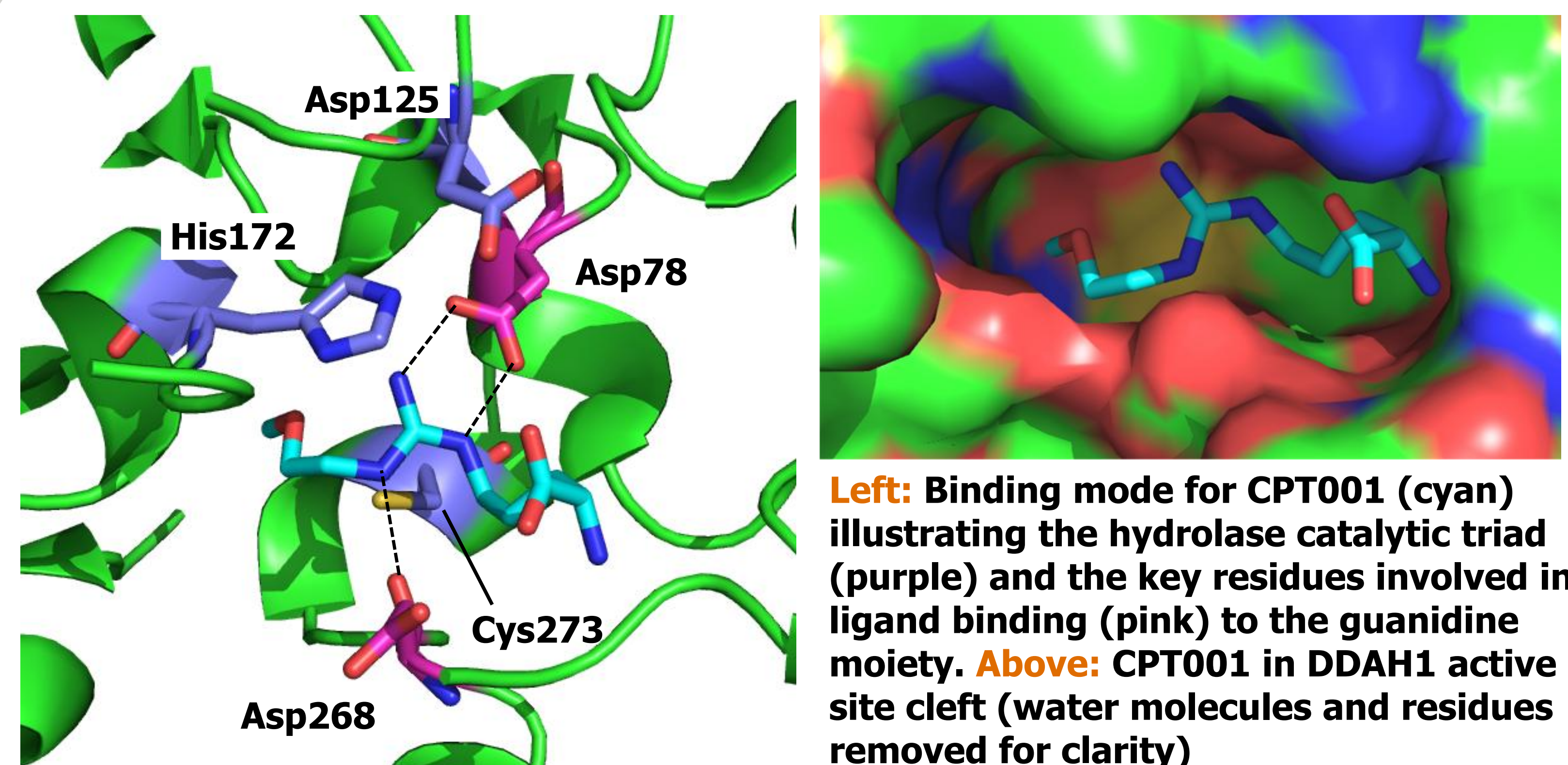
- Two human isoforms of DDAH (DDAH1 and DDAH2) have been identified that have restricted tissue distribution. These approximately correspond to those of nNOS (neuronal NOS) and eNOS (endothelial NOS) respectively, suggesting an isoform-specific mechanism for regulating NOS activity
- Selectivity of a DDAH1 inhibitor over the various NOS enzymes is critical for any therapeutic agent for sepsis. Selectivity over DDAH2 is also preferred

3. Strategy and Inhibitor Synthesis

- An X-ray structural platform was established for the determination of inhibitor:human DDAH1 structures at high resolution. This allowed a thorough examination of enzyme:inhibitor interactions for both covalent and non-covalent inhibitors
- Novel ligands were designed based around the structure of NMMA with modifications to the guanidine moiety. It was envisaged that these analogues would disrupt the hydrolysis mechanism of DDAH1³
- Inhibitors were designed to be "arginine-like" to take advantage of active uptake processes and to fill the narrow DDAH1 binding pocket

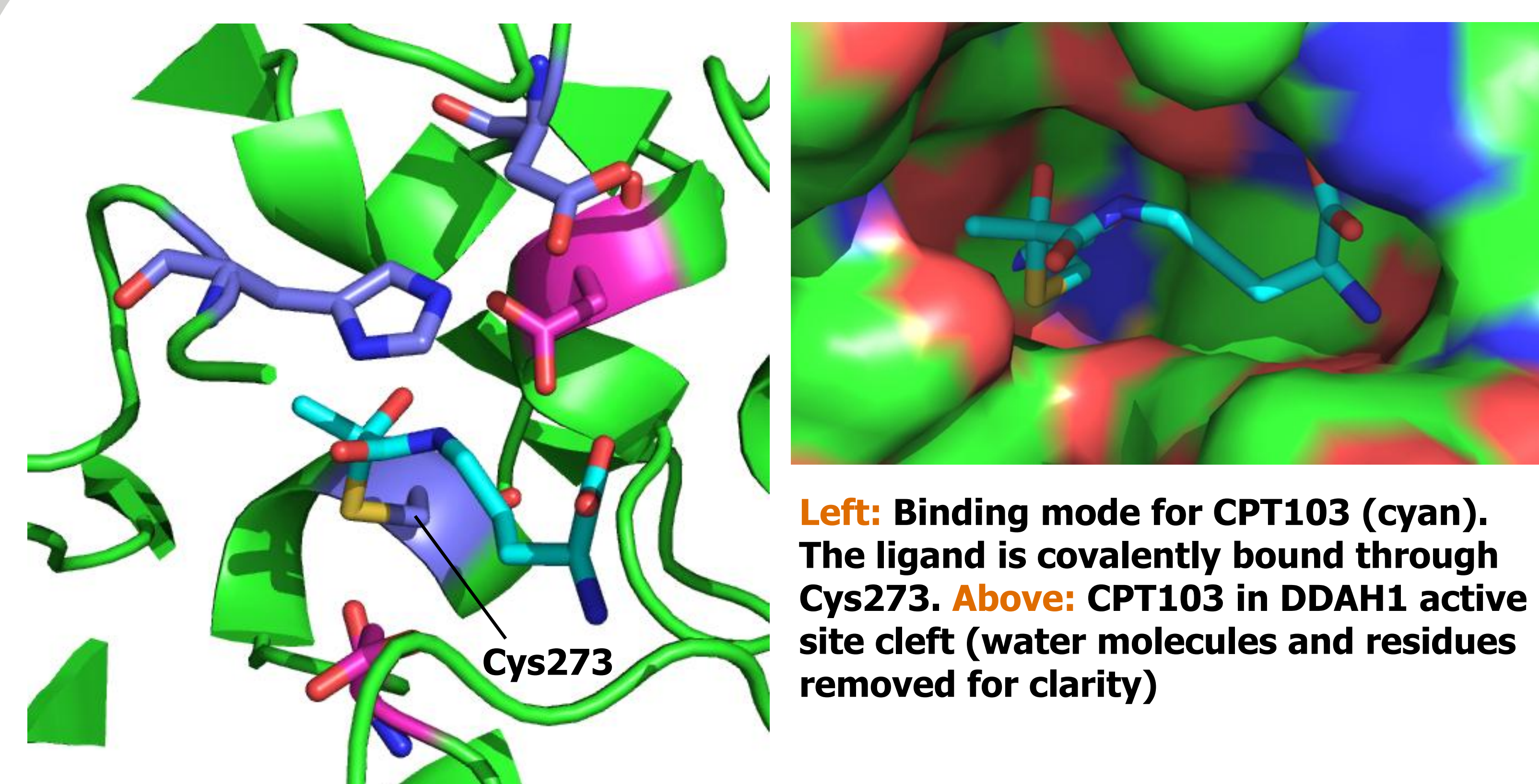


4. CPT001 - Binding Mode 1



- Key hydrogen bonding interactions were observed between the guanidine moiety of CPT001 and the carboxyl group of Asp78 and the backbone carbonyl group of Asp268
- CPT001 bound to human DDAH1 showed an "S"-shaped conformation of the inhibitor and filled the narrow binding pocket. The methoxyethyl group was found to be optimal for binding
- CPT001 was found to inhibit DDAH1 activity *in vitro* – IC₅₀ value of 20 μM¹

5. CPT103 - Binding Mode 2



- CPT103 possesses an electrophilic α-keto amide group and was designed to be a covalent inhibitor
- X-ray data shows a covalent bond from Cys273 to the ketone carbon. Typical substrate hydrolysis occurs at the guanidine carbon of NMMA and ADMA
- Selectivity for DDAH1 over DDAH2 is predicted to be poor - further derivatisation of the α-keto group offers potential to improve selectivity

6. Conclusions

- Distinct covalent and non-covalent binding modes identified. CPT001 had a different conformation to CPT103, whose binding mode closely resembled that of citrulline
- Variation of the "tail" group has potential to improve potency and selectivity over DDAH2
- CPT001 is selective for DDAH1 over NOS isoforms.¹ Selectivity screening of CPT103 is planned
- The production of NO can be reduced indirectly by increasing levels of the endogenous inhibitors, NMMA and ADMA



Acknowledgments

This work was funded by the Wellcome Trust Seeding Drug Discovery Initiative, Grant 084301/Z/07/Z