

# Identification and Characterisation of Novel Substrates and Binding Partners of the Asparaginyl Hydroxylase, FIH

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# Abstract

The ability of cells to sense and respond to sub-optimal levels of oxygen is a key requirement for organism survival. Many cellular and physiological signalling pathways have been identified as being sensitive to oxygen levels, although remarkably, few of these pathways have been successfully linked with a genuine “oxygen sensing” molecule. Discovery of the 2-oxoglutarate-dependent asparaginyl hydroxylase, Factor Inhibiting HIF (FIH), as an oxygen sensitive regulator of the Hypoxia-inducible Factor (HIF) transcription factors has therefore led to considerable interest in the enzyme as a potential regulator of multiple oxygen-regulated processes. In this work, the known substrate repertoire of FIH was expanded using both yeast 2-hybrid (Y2H) and bioinformatics-based approaches. Potential positives identified in the Y2H screen included a number of proteins which contain an ankyrin repeat structural domain (ARD), and subsequent characterisation of these proteins by *in vitro* hydroxylation assay suggest that both Fetal Globin Inducing Factor (FGIF) and Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit B (PP6-ARS-B) are both novel substrates of FIH. Concomitant with this discovery, a number of other ARD-containing proteins have been reported as substrates of FIH in the literature, thus suggesting that hydroxylation of ARDs by FIH is common. Comparison of the target sites in these substrates reveals an “FIH preferred sequence” of LXXXXX[-]φN, however, it was discovered that FIH can also bind an ARD constrained in its folded state, suggesting that the tertiary fold of ankyrins may also participate in enzyme recruitment. Thus far, hydroxylation of ARDs reported in the literature has not been found to have a significant functional effect on ARD biology. Largely consistent with this, an assessment of the influence of FIH-mediated hydroxylation on IκBα stability and interaction with NFκB in this work suggested that the modification has only subtle effects. Furthermore, FIH was found to have no clear effect on the methyltransferase activity of the novel ARD-containing substrate, G9a. In addition to ARD-containing proteins, the Y2H also identified a number of non-ARD-containing proteins which displayed weak interactions with FIH that were inducible by the FIH inhibitor, DMOG. *In vitro* hydroxylation assays suggest that these proteins are not FIH substrates, and further study will be required to establish the biological significance of these interactions. Overall, this work suggests that FIH interfaces with many partners, and it remains to be determined how these interactions influence the function of FIH, as well as that of its substrates and binding proteins.



# Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Since this is the part of the thesis that EVERYONE reads, I'd better do a decent job (and since I'm writing it at 1 in the afternoon, instead of at 3 in the morning (like my honours thesis), hopefully it will be a little more comprehensible).

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# Author contributions


The following author contribution statement details the contribution of each author to the published article, “Consequences of IkappaB alpha hydroxylation by the factor inhibiting HIF (FIH)”, which can be found in section 6.2.



# Statement of Authorship

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Melinda Mulvihill	Planned experiments, generated data for Figs 4, 5 and 6, interpreted experiments, discussed data.
Vera Alverdi	Planned experiments, generated data for Fig 2, interpreted experiments.
Daniel Peet	Co-conceived the project, planned experiments, interpreted experiments, discussed data, edited the paper.
Elizabeth Komives	Co-conceived the project, planned experiments, interpreted experiments, discussed data, wrote and edited the paper, acted as corresponding author.

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## Co-1st Author

Name of Co-1st Author (Candidate)	Rachel Hampton-Smith		
Contribution to the Paper	Co-conceived the project, planned experiments, generated data, interpreted experiments, discussed data, edited the paper.		
Overall percentage (%)	20%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the joint primary author of this		
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## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
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- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Contribution to the Paper
Ingrid Devries	Planned experiments, generated data for Fig 3, interpreted experiments, discussed data.
Melinda Mulvihill	Planned experiments, generated data for Figs 4, 5 and 6, interpreted experiments, discussed data.
Vera Alverdi	Planned experiments, generated data for Fig 2, interpreted experiments.
Daniel Peet	Co-conceived the project, planned experiments, interpreted experiments, discussed data, edited the paper.
Elizabeth Komives	Co-conceived the project, planned experiments, interpreted experiments, discussed data, wrote and edited the paper, acted as corresponding author.

**Daniel Peet**

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# Statement of Authorship

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