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Decoding Heme-Gαi Protein Crosstalk with Peptide Tools

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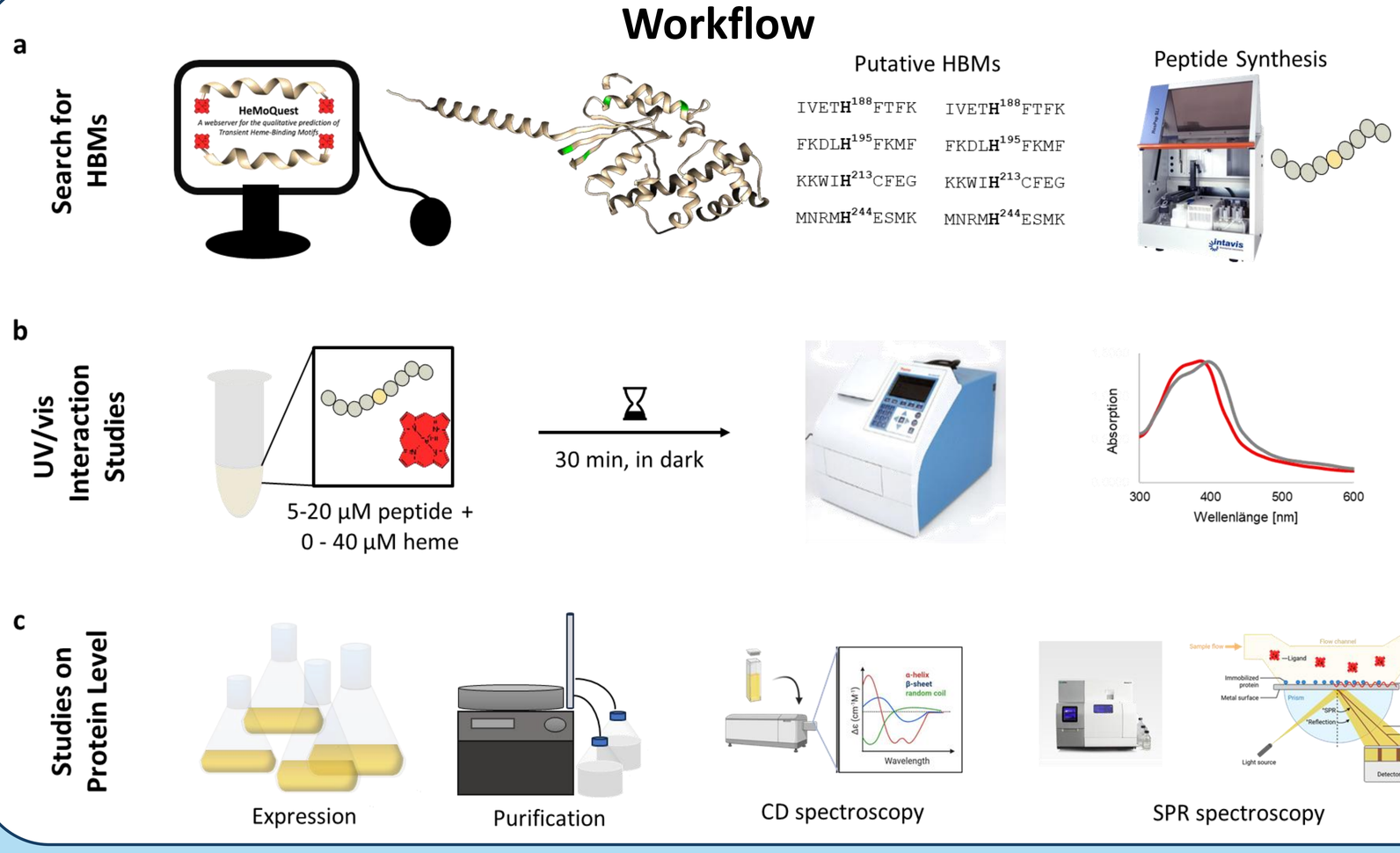
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Background

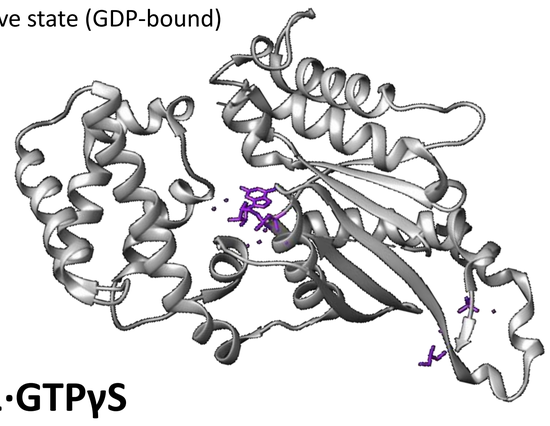
Heme is an iron-containing porphyrin that serves as an essential prosthetic group in proteins involved in oxygen transport, electron transfer, and catalysis. Beyond these classical functions, **heme can also act as a signaling molecule**. Recent evidence suggests that extracellular heme influences **G protein-coupled receptor (GPCR) signaling** in mouse models of sickle cell disease and intravascular hemolysis.[1] However, whether heme can **interact directly with G proteins** and modulate their signaling remains poorly understood. Gα proteins contain functional heme-binding motifs (HBMs) that allow direct heme binding and influence G-protein structure and activity. The aim is to characterize **heme binding to Gα proteins** and its consequences for **G-protein activity and function**.

Workflow



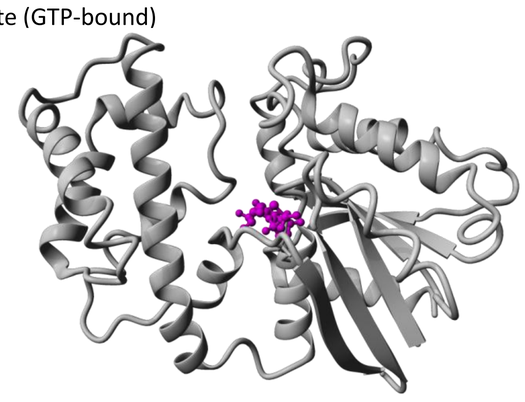
Gαi · GDP

inactive state (GDP-bound)



Gαi1·GTPγS

active state (GTP-bound)



PDB: 3UMR[6]. (purple: nucleotide)

Results & Discussion

HeMoQuest and *in silico* investigations identified potential heme-binding sites in Gαi. The corresponding motifs were synthesized by solid-phase peptide synthesis (SPPS) and analyzed for heme binding by UV/vis spectroscopy (Fig. 3, Tab. 1).

Sequence	Binding Affinity (K_D)
IVETH188FTFK	n.b
FKDLH195FKMF	12.5 µM
KKWIH213CFEG	5.2 µM
MNRMH244ESMK	36.5 µM

Table 1. UV/vis data for heme-binding to HBMs within Gαi.

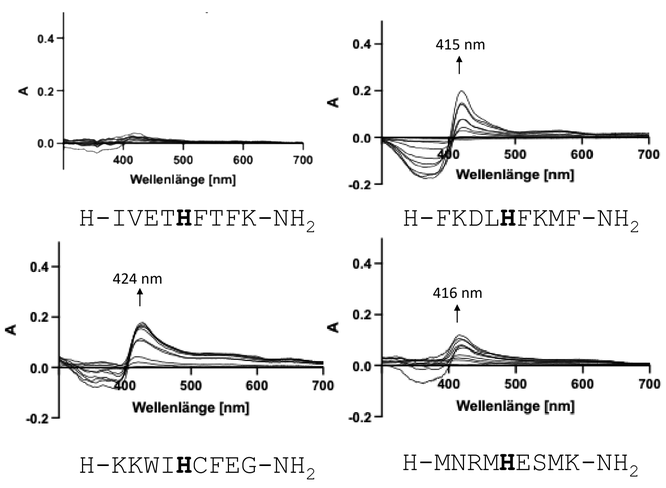


Figure 3. UV/vis difference spectra of HBMs in Gαi.

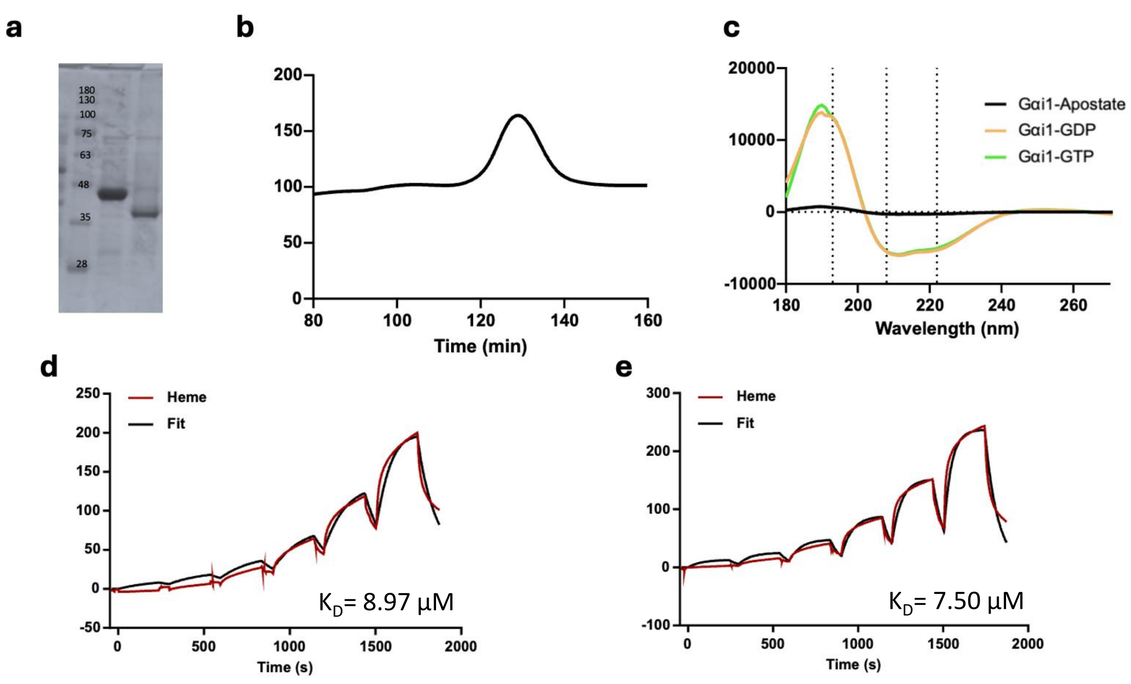


Figure 4. Overview of protein level studies. (a) 10%-SDS-Gel of Gαi before and after His_6-tag cleavage. (b) Chromatogram of size-exclusion chromatography of Gαi purification. (c) CD spectroscopy results of His_6-tag cleaved Gαi. (d,e) SPR sensograms of heme binding to Gαi-GDP and Gαi-GMPPNP, respectively.

References

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Conclusions & Outlook

- Gαi1 contains specific, surface-exposed HBMs.
- Predicted interactions were validated at the peptide and the protein level.
- Heme-G protein complex formation can affect Gαi catalytic activity.
- Further studies will explore the underexplored mechanisms by which heme may modulate GPCR/G-protein signaling in blood cells.

Acknowledgements

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