applied sciences

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Article

Tracking heme-protein interactions in healthy and pathological human serum in native conditions by miniaturized FFF-multi-detection

Valentina Marassi [1,2*§], Stefano Giordani [1§], Pierluigi Reschiglian [1,2], Barbara Roda[1,2] and Andrea Zattoni [1,2*]

[1] Department of Chemistry G. Ciamician, University of Bologna, Bologna, Italy
[2] byFlow srl, Bologna, Italy
* Correspondence: valentina.marassi2@unibo.it; andrea.zattoni@unibo.it
§ The two authors contributed equally

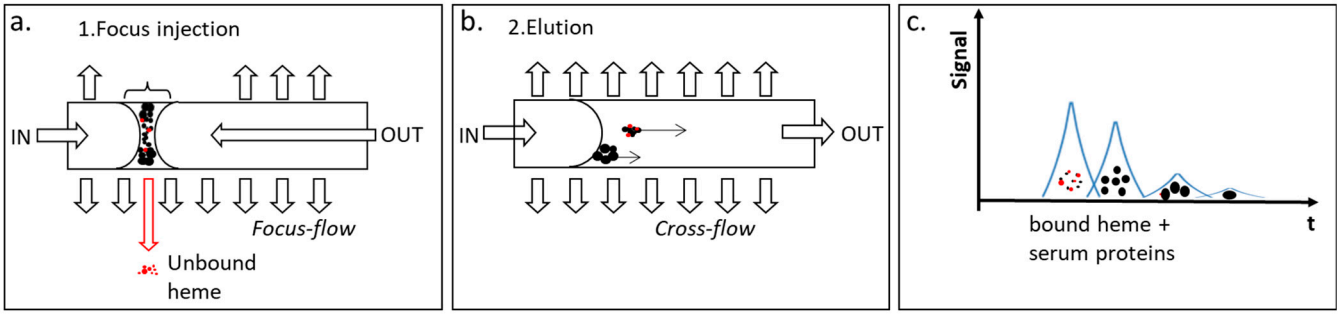

Figure S1. Platform schematics and method output. a) focus-injection: unbound heme is filtered away from the channel and does not interfere with the analysis. b) Elution: species are separated by size and reach the detectors. c) Profile output: different proteins are eluted at different times and are characterized by specific protein and heme absorption. Bound heme is detected peak by peak.

Injections of the same heme amount used in the serum/protein-heme mixtures (plus an additional analysis using heme 100 μM) were performed to evaluate recovery and ensure the absence of unbound heme contribution to the profiles (Figure S2). FIA analyses (see Materials and Methods) show a concentration-dependent signal (grey profiles), while the signal obtained with the separation method (red) was flat even at the highest concentration used. This confirms the total free-heme filtration and the selective detection of bound heme in the separation profiles.

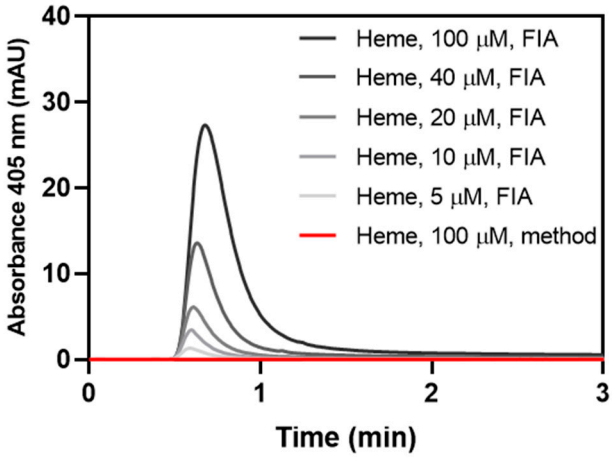

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Figure S2. Negative control of heme alone in in HF5-multidetection.