Supplementary Information (SI) for Lab on a Chip.
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Supporting Information

This supporting information section provides supplementary data and detailed analysis of the performance of the biosensing detection platform described in the main text. Figure S1 shows representative images of the PS@Se heterodimer probes after binding to the target proteins S100B, GFAP, and UCHL1 at six concentration gradients from 1 ng/mL to 100 ng/mL. The images were acquired using four imaging devices, including a scanning electron microscope (SEM), a Nikon upright microscope equipped with a CCD camera, a smartphone mounted on the eyepiece of a standard optical microscope (as an alternative to a professional CCD camera), and a portable clip-on smartphone microscope, visually demonstrating the detection morphology under different imaging modalities.

To systematically evaluate the accuracy and bias of the detection method across concentration levels, calibration residual plots were generated (Figure S2). The analysis revealed that for both the standard (A) and the target proteins in clinical samples (S100B, GFAP, and UCH-L1; B-D), the variability (dispersion) of the prediction residuals increased with the analyte concentration, indicating evident heteroscedasticity. This pattern suggests that the precision of the assay, as measured by residual variability, is concentration-dependent, with the absolute error range widening in the high-concentration interval. Importantly, all data points were randomly distributed around the zero baseline without systematic over- or under-estimation bias. This result quantitatively characterizes the error structure of the method, indicating that its concentration-dependent precision should be considered when quantifying high-concentration samples in future applications.

Table S2 summarizes the analytical performance of the developed biosensor across a six-order-of-magnitude concentration range (from 1 to 100,000 pg/mL) for both recombinant protein standards and clinical samples. For the standard proteins, the biosensor demonstrated good accuracy and precision, with most recoveries between 95% and 115% and coefficients of variation (CVs) largely below 25%. However, a notable mean negative bias (e.g., approximately -6,200 pg/mL for S100B and UCH-L1) was observed at the highest concentration (100,000 pg/mL), suggesting potential signal saturation at extremely high analyte levels.

Analysis of clinical samples revealed increased performance variability, particularly at the lower concentration decades. The higher CVs at low concentrations likely stem from the analyte concentrations approaching the detection limit of the biosensor, where a reduced signal-to-noise ratio amplifies the impact of random error on measurement repeatability. Distinct performance patterns were evident across different biomarkers and sample types. For instance, measurements in clinical saliva samples often showed positive biases, whereas detection of UCH-L1 in clinical samples consistently yielded negative biases. This indicates potential differences in detection specificity or antibody cross-reactivity for different proteins within different biological matrices. We conducted a study comparing standard quantification under simulated hemolytic conditions (Table S3), yet the underlying mechanisms require further investigation.

Notably, numerous entries in the table are marked as "Not Applicable (NA)". This is primarily due to two reasons: first, a lack of available clinical samples at specific high concentration decades (e.g., ≥10,000 pg/mL) for certain sample types like blood, urine, and saliva; second, a substantial portion of clinical samples had ELISA values below the assay's limit of detection in the low concentration range, resulting in an insufficient number of low-concentration samples with valid reference data for comparison. For example, at 1 pg/mL, the recovery for GFAP in clinical samples was as low as 58.98% ± 23.47% with a mean difference of -6.54 pg/mL, and this calculation was based on only 4 samples. At the same concentration level, only a single urine sample was available. This sparseness of clinical sample data at extreme concentrations limited a more robust assessment of the biosensor's low-end performance based solely on clinical correlation. It should be noted that the limit of detection (LOD = 1 pg/mL) stated in this study was not derived from a formal LoD/LOQ study conducted strictly per guidelines such as CLSI EP17 or IUPAC. This value was obtained from previous experimental investigations, intended to represent the minimum protein concentration reliably detectable under our experimental conditions. Additionally, in the present study, we supplemented the analysis with 5 replicate measurements for each of the three recombinant biomarker proteins (S100B, GFAP, and UCH-L1) at concentrations of 1, 2, and 5 pg/mL (Table S3).

This study conducted a comprehensive assessment of the analytical performance of the biosensing detection platform (Table S3). Table S3 includes: low concentration limit of detection verification (5 replicate measurements for each standard at concentrations of 1, 2, and 5 pg/mL); hemolysis interference test (5 measurements each for S100B, GFAP, and UCHL1 at 1,000 pg/mL in commercially available standard serum spiked with 1.0 g/L hemoglobin to simulate moderate hemolysis); between-batch precision (measurements for each protein at 1,000 pg/mL with 5 chips per batch, across 3 different batches); within-batch precision (10 replicate measurements for each protein at

1,000 pg/mL using chips from the same batch); inter-operator precision (5 measurements for each protein at 1,000 pg/mL by three different operators using chips from the same batch); and chip storage stability (5 replicate measurements for each protein at 1,000 pg/mL on days 0, 1, 3, 5, and 7 after chip preparation). The overall recovery for the low concentration (1, 2, 5 pg/mL) verification was 122.51% ± 22.07%.

The overall recovery for the hemolysis interference test was 94.32% ± 33.70%, indicating that simulated moderate hemolysis indeed introduced some interference, the mechanism of which requires further elucidation in future studies; potential interferences such as hyperlipidemia, bilirubin, and heterophilic antibodies are also limitations of this platform, and more comprehensive interference experiments are needed in the future. Considering that this study represents the validation stage of a prototype for a portable point-of-care testing device, the platform demonstrates acceptable precision: overall between-batch agreement was 94.64% ± 31.63% (1,000 pg/mL), overall within-batch agreement was 95.35% ± 29.92% (1,000 pg/mL), overall inter-operator agreement was 95.92% ± 31.25% (1,000 pg/mL), with results for the three independent operators being 96.15% ± 31.55%, 96.01% ± 31.80%, and 95.60% ± 32.61%, respectively.

Our previous work on analogous printed biosensor platforms demonstrated stability over a two-week period under ambient storage[1, 2]. The new data from this study show the following stability of the chips under room temperature storage: overall recovery on day 0 was 97.27% ± 27.54%, day 1 was 95.53% ± 25.57%, day 3 was 93.31% ± 23.51%, day 5 was 90.37% ± 19.99%, and day 7 was 73.82% ± 41.96% (1,000 pg/mL). We have discussed potential measures to extend the shelf life in the main text Discussion section, which await further investigation.

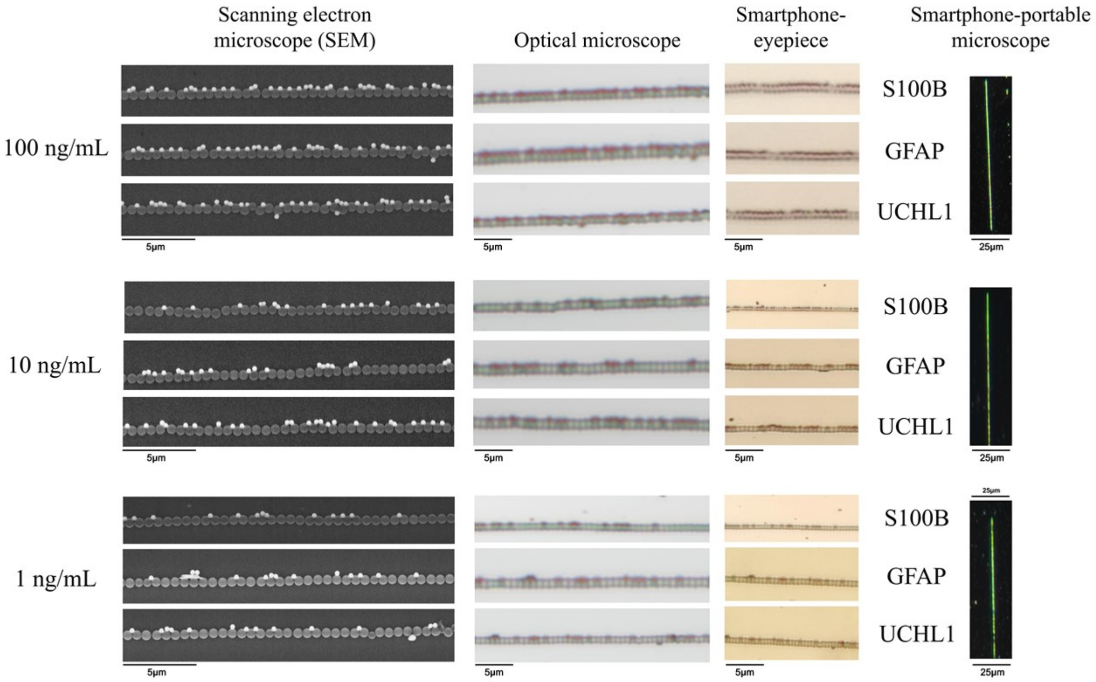

Figure S1: Representative images of PS@Se heterodimers bound to S100B, GFAP, and UCHL1 at three concentrations spanning from 1 ng/mL to 100 ng/mL.

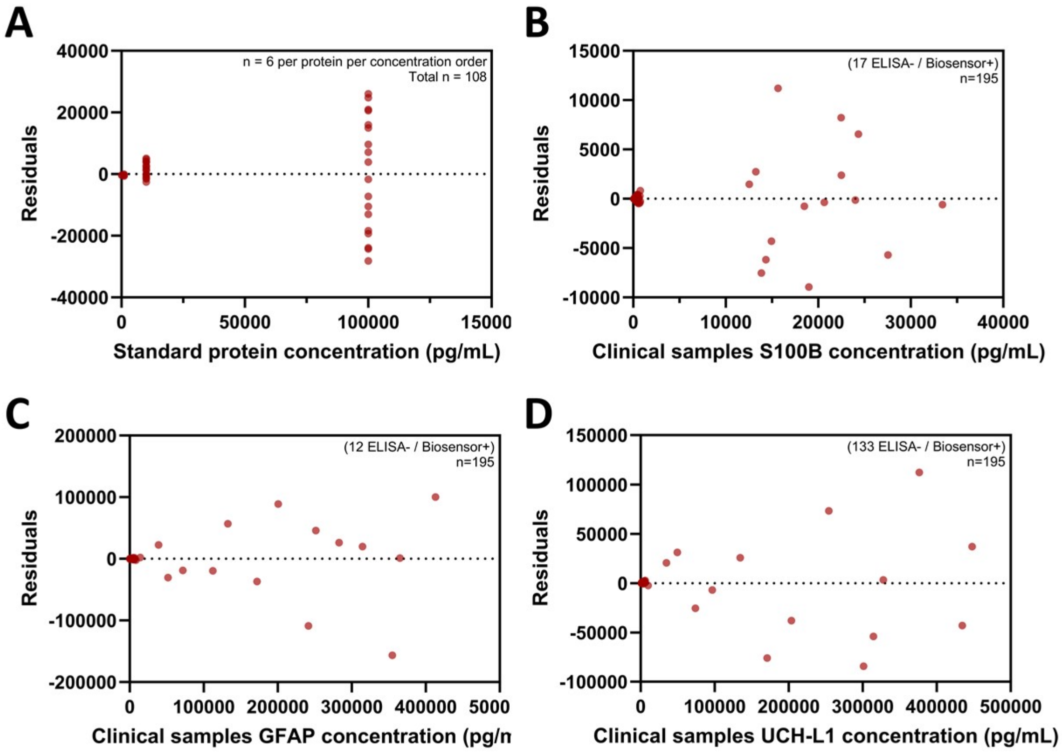

Figure S2: Calibration residual plots. The x-axis represents concentration (pg/mL), and the y-axis represents prediction error: (A) External validation using standard protein (versus the actual concentration). (B) External validation of S100B protein in clinical samples (versus ELISA). (C) External validation of GFAP protein in clinical samples (versus ELISA). (D) External validation of UCH-L1 protein in clinical samples (versus ELISA).

Table S1: The demographic and clinical characteristics of the study cohort. Participants (N=75): 60 TBI patients (45 with blood/urine/saliva; 15 with CSF) and 15 healthy controls (blood/urine/saliva). Total samples: 195.

(A) Patient-level characteristics of the TBI patients. Excluding Healthy Controls, only 60 TBI patients were included; retained at the 0% level.

Category	**Variable**	**Overall**	**Mild (GCS 13–15)**	**Moderate (GCS 9–12)**	**Severe (GCS ≤8)**
Sampling volume	Total patients, n	60	15	15	30
Demographics	Age (years), median [IQR]	29.5 [22-43]	33 [24.5-44]	34 [23-41.5]	27 [20-40]
	Sex, male n (%)	31 (52%)	10 (67%)	11 (73%)	10 (33%)
	Sex, female n (%)	29 (48%)	5 (33%)	4 (27%)	20 (67%)
Injury severity	GCS at presentation, median [IQR]	8 [5-12.25]	14 [13–15]	10 [9–11]	5 [5–6]
Injury mechanism	Fall, n (%)	28 (47%)	6 (40%)	7 (47%)	15 (50%)
	Motor vehicle collision, n (%)	24 (40%)	5 (33%)	5 (33%)	14 (47%)
	Assault/blunt, n (%)	6 (10%0	3 (20%)	3 (20%)	0 (0%)
	Other/unknown, n (%)	2 (3%)	1 (7%)	0 (0%)	1 (3%)
Imaging / diagnosis	CT performed, n (%)	60(100%)	15 (100%)	15 (100%)	30 (100%)
	ICH on CT, n (%)	31 (52%)	3 (20%)	5 (33%)	23 (77%)
	No ICH on CT, n (%)	29 (48%)	12 (80%)	10 (67%)	7 (23%)
Key clinical context	Time from injury to first sample (h), median [IQR]	4 [2.5–5.5]	4 [2.75–5.75]	4 [2.75–4.75]	4 [2.75–5.5]

Note: *IQR, Interquartile Range; GCS, Glasgow Coma Scale; ICH, Intracerebral Hemorrhage.*

(B) Sample-level overview. Total N = 195 samples from 75 individuals, comprising 60 TBI patients and 15 healthy controls; CSF was collected during surgery; the sampling time window excluded Healthy Controls and patients with CSF collection only, n=45 TBI patients; severity distribution excludes Healthy Controls, , n=60 TBI patients; retained at the 0% level.

Category	**Variable**	**Overall**
Sampling volume Biofluid samples distribution N=75 individuals	Total samples, n	195
	Total Serum, n (%)	60 (31%)
	Total Urine, n (%)	60 (31%)
	Total Saliva, n (%)	60 (31%)
	Total CSF, n (%)	15 (8%)
Sampling time window (post-injury, h) N=45 TBI patients	Time from injury to sampling (h), median [IQR] (min–max)	3.9 [2.5-5.5] (0.5–6.0)
	0–2 h, n (%)	6 (13%)
	>2–4 h, n (%)	21 (47%)
	>4–6 h, n (%)	18 (40%)
Matrix × severity N=60 TBI patients	Serum samples in mild/moderate/severe TBI, n	15 / 15 / 15
	Urine samples in mild/moderate/severe TBI, n	15 / 15 / 15
	Saliva samples in mild/moderate/severe TBI, n	15 / 15 / 15
	CSF samples in mild/moderate/severe TBI, n	0 / 0 / 15

***Note:** IQR, Interquartile Range; CSF, Cerebrospinal Fluid.*

Table S2: Analytical performance of the biosensor across concentration decades for recombinant standards and clinical samples. Mean Difference is reported as biosensor value minus actual value (for standards) or ELISA value (for clinical samples). Positive values indicate biosensor readings are higher than reference, negative values indicate lower. Clinical sample data are based on biosensor-detected concentration decades and include only samples with ELISA measurements ≥ ELISA LOD.

Concentration Decade (pg/mL)		**100,000**		**10,000**		**1,000**		**100**		**10**		**1**	
Sample Type	**Biomarker**	**Recovery±CV (%)**	**Mean Difference (pg/mL)**	**Recovery±CV (%)**	**Mean Difference (pg/mL)**	**Recovery±CV (%)**	**Mean Difference (pg/mL)**	**Recovery±CV (%)**	**Mean Difference (pg/mL)**	**Recovery±CV (%)**	**Mean Difference (pg/mL)**	**Recovery±CV (%)**	**Mean Difference (pg/mL)**
Standard Protein	**Total**	95.36% ± 19.35%	-4635.28	114.19% ± 20.53%	1418.72	97.73% ± 20.88%	-22.66	111.68% ± 25.49%	11.68	95.78% ± 20.82%	-0.42	115.00% ± 33.11%	0.15
	S100B	93.83% ± 22.23%	-6175.00	111.46% ± 26.99%	1146.00	96.09% ± 24.70%	-39.12	110.68% ± 27.00%	10.68	94.67% ± 24.20%	-0.53	115.00% ± 25.05%	0.15
	GFAP	98.44% ± 19.90%	-1555.83	117.30% ± 21.11%	1729.83	101.12% ± 21.47%	11.18	118.17% ± 26.82%	18.17	99.67% ± 21.44%	-0.03	126.67% ± 36.58%	0.27
	UCH-L1	93.83% ± 19.11%	-6175.00	113.80% ± 15.94%	1380.33	96.00% ± 19.77%	-40.03	106.20% ± 26.12%	6.20	93.00% ± 19.89%	-0.70	103.33% ± 39.03%	0.03
Clinical Sample	**Total**	101.87% ± 27.51%	-1508.44	112.41% ± 34.59%	-6124.92	110.89% ± 29.90%	-166.65	123.22% ± 27.96%	-45.76	103.16% ± 25.72%	-1.84	58.98% ± 23.47%	-6.54
	S100B	NA	NA	120.04% ± 26.48%	4,017.48	162.32% ± 44.00%	-787.97	128.77% ± 25.46%	63.68	102.02% ± 25.11%	-1.16	NA	NA
	GFAP	110.26% ± 28.04%	23,949.03	105.28% ± 47.92%	-2,374.57	116.94% ± 27.35%	342.11	121.31% ± 28.07%	45.39	104.59% ± 26.61%	-2.52	58.98% ± 23.47%	-6.54
	UCH-L1	91.63% ± 23.31%	-26,965.91	96.76% ± 52.30%	-20,017.66	99.87% ± 21.83%	-54.08	78.70% ± 27.82%	-246.37	NA	NA	NA	NA
	Serum	NA	NA	NA	NA	115.29% ± 31.02%	154.45	116.47% ± 32.21%	1,199.12	99.27% ± 23.71%	-5.04	61.63% ± 25.41%	-5.77
	Urine	NA	NA	NA	NA	110.69% ± 19.10%	74.91	132.45% ± 27.43%	72.26	105.67% ± 28.43%	-1.51	51.03%[*]	-8.83[*]

Concentration Decade (pg/mL)		100,000		10,000		1,000		100		10		1	
Sample Type	**Biomarker**	**Recovery ± CV (%)**	**Mean Difference (pg/mL)**	**Recovery ± CV (%)**	**Mean Difference (pg/mL)**	**Recovery ± CV (%)**	**Mean Difference (pg/mL)**	**Recovery ± CV (%)**	**Mean Difference (pg/mL)**	**Recovery ± CV (%)**	**Mean Difference (pg/mL)**	**Recovery ± CV (%)**	**Mean Difference (pg/mL)**
	Saliva	NA	NA	NA	NA	97.95% ± 28.79%	51.77	121.01% ± 21.03%	43.32	104.55% ± 24.60%	1.41	NA	NA
	CSF	101.87% ± 27.51%	1,037.31	112.41% ± 34.59%	-2,319.21	94.66% ± 46.45%	-1,727.49	NA	NA	NA	NA	NA	NA

Notes: *NA = Not Applicable (no data available for this study). CSF = Cerebrospinal Fluid. * = Only one sample exists in this case.*

Table S3: Analytical performance evaluation of the assay for S100B, GFAP, and UCHL1 proteins. Mean Difference is reported as biosensor value minus actual value (for standards) . Positive values indicate biosensor readings are higher than reference, negative values indicate lower.

Protein		**Total**		**S100B**		**GFAP**		**UCH-L1**	
Group		**Recovery ± CV (%)**	**Mean Difference (pg/mL)**	**Recovery ± CV (%)**	**Mean Difference (pg/mL)**	**Recovery ± CV (%)**	**Mean Difference (pg/mL)**	**Recovery ± CV (%)**	**Mean Difference (pg/mL)**
Low-Concentration Range Test	**Total**	122.51% ± 22.07%	0.54	122.27% ± 20.48%	0.53	126.73% ± 23.37%	0.69	118.53% ± 23.22%	0.42
	5 pg/mL	119.20% ± 22.28%	0.96	118.80% ± 22.07%	0.94	127.20% ± 22.86%	1.36	111.60% ± 25.07%	0.58
	2 pg/mL	119.00% ± 22.86%	0.38	116.00% ± 22.06%	0.32	117.00% ± 26.62%	0.34	124.00% ± 24.43%	0.48
	1 pg/mL	129.33% ± 21.57%	0.29	132.00% ± 19.61%	0.32	136.00% ± 23.60%	0.36	120.00% ± 24.30%	0.20
Hemolysis Interference Test	**1000 pg/mL**	94.32% ± 33.70%	-56.79	88.07% ± 36.51%	-119.3	88.69% ± 33.52%	-113.07	106.20% ± 31.27%	62.01
Inter-Lot Test	**Total**	94.64% ± 31.63%	-53.60	87.88% ± 35.15%	-121.23	98.78% ± 31.59%	-12.18	97.26% ± 29.33%	-27.37
	Batch 1	93.69% ± 31.66%	-63.11	91.24% ± 34.59%	-87.62	100.63% ± 31.36%	6.32	89.20% ± 35.38%	-108.02
	Batch 2	101.05% ± 30.28%	10.47	98.60% ± 32.00%	-14.02	107.99% ± 29.22%	79.92	96.55% ± 35.75%	-34.48
	Batch 3	89.18% ± 34.08%	-108.15	73.79% ± 41.61%	-262.06	87.72% ± 38.70%	-122.78	106.04% ± 20.74%	60.38
Intra-Assay Test	**1000 pg/mL**	95.35% ± 29.92%	-46.53	95.89% ± 31.43%	-41.13	76.99% ± 33.36%	-230.09	113.16% ± 15.99%	131.64
Inter-Operator Test	**Total**	95.92% ± 31.25%	-40.83	84.27% ± 30.30%	-157.31	95.88% ± 39.60%	-41.25	107.61% ± 19.47%	76.01
	Operator 1	96.15% ± 31.55%	-38.53	84.54% ± 25.31%	-154.56	89.14% ± 45.45%	-108.6	114.76% ± 18.65%	147.56
	Operator 2	96.01% ± 31.80%	-39.92	77.25% ± 27.70%	-227.48	102.89% ± 39.38%	28.92	107.88% ± 20.46%	78.80

Protein		Total		S100B		GFAP		UCH-L1	
Group		Recovery ± CV (%)	Mean Difference (pg/mL)	Recovery ± CV (%)	Mean Difference (pg/mL)	Recovery ± CV (%)	Mean Difference (pg/mL)	Recovery ± CV (%)	Mean Difference (pg/mL)
	Operator 3	95.60% ± 32.61%	-44.03	91.01% ± 38.81%	-89.88	95.59% ± 42.39%	-44.06	100.18% ± 21.38%	1.84
Time-Stability Test	**Total**	90.06% ± 28.42%	-99.40	90.18% ± 27.28%	-98.23	93.16% ± 28.43%	-68.38	86.84% ± 30.30%	-131.6
	0 day	97.27% ± 27.54%	-27.28	91.20% ± 34.57%	-87.98	100.59% ± 31.36%	5.92	100.02% ± 21.26%	0.22
	1 day	95.53% ± 25.57%	-44.69	94.76% ± 22.80%	-52.4	95.52% ± 28.50%	-44.84	96.32% ± 30.78%	-36.84
	3 day	93.31% ± 23.51%	-66.87	87.18% ± 23.44%	-128.16	98.21% ± 20.81%	-17.9	94.54% ± 29.35%	-54.56
	5 day	90.37% ± 19.99%	-96.33	88.74% ± 21.38%	-112.62	94.68% ± 20.40%	-53.2	87.68% ± 22.04%	-123.16
	7 day	73.82% ± 41.96%	-261.85	89.00% ± 41.26%	-109.98	76.81% ± 45.80%	-231.9	55.63% ± 13.80%	-443.66

Reference

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