APPLICATION NOTE

From Nanomolar Binding to Neat Plasma: Surface-free Mini Protein Characterization

Peptomyc profiles Omomyc variants affinity, specificity, and non-specific binding in plasma in a single workflow on the Fluidity One-M.

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At a glance

THE PROBLEM

Characterizing DNA-targeted mini-protein therapeutics requires answers to three questions: 1) variant affinity, 2) sequence specificity, and 3) non-specific binding in physiological matrices. No single conventional platform answers all three. Today this requires fragmented workflows across SPR, EMSA, and separate stability assays, and the plasma question often goes unanswered entirely.

HOW THE FLUIDITY ONE-M ANSWERED ALL THREE

- **Affinity:** K_D from 69.8 nM to 7,490 nM (>100-fold range) resolved in one in-solution workflow
- **Specificity:** E-box sequence discrimination vs. non-specific DNA confirmed across all three Omomyc variants
- **Plasma profiling:** Non-specific binding profiled from PBS-T through 100% mouse plasma without reformatting or diluting the assay
- **Sample size:** 4 µL per datapoint
- **Time:** < 25 min per binding curve
- **Immobilization:** None required
- **Purification:** None required

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Abstract

Scientists developing DNA-targeted mini-protein therapeutics need quantitative answers to three characterization questions: What is the binding affinity across a variant panel? Does binding discriminate the target DNA sequence? And how does the molecule behave in physiological matrices like undiluted plasma? No single conventional biophysical platform can answer all three. SPR and ELISA address affinity but require immobilization and fail in complex matrices. Circular dichroism and EMSA offer qualitative confirmation but not quantitative K_D. Non-specific binding in plasma typically goes uncharacterized.

The Fluidity One-M, powered by Microfluidic Diffusional Sizing (MDS), answers all three in a single in-solution workflow. In this study, Peptomyc's team used the Fluidity One-M to characterize Omomyc mini-protein variants generated through pipeline development efforts to further study their lead molecule OMO-103 (Phase 1 trial published in *Nature Medicine*). They resolved DNA-binding affinities from 69.8 nM to 7,490 nM across three variants, confirmed E-box sequence discrimination, and profiled non-specific binding from buffer through 100% mouse plasma. Each measurement required 4 µL of sample and completed in under 25 minutes, with no immobilization, no purification, and no buffer restrictions.

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Introduction: The Characterization Gap in Mini-Protein Therapeutics, Solved by Fluidity One-M

OMO-103, a mini-protein inhibitor of the MYC oncogene, demonstrated clinical activity in a Phase 1 solid tumor trial published in *Nature Medicine* (Garralda et al., 2024) [1]. The Omomyc variants developed alongside that program exemplify a broader challenge in mini-protein therapeutics: the biophysical tools available for characterization cannot keep pace with the molecules being developed. Mini-proteins combine the target specificity of biologics with the favorable pharmacokinetics of small molecules [2, 3], but their small size, structural dynamics, and DNA-binding mode create problems for the platforms most labs rely on.

Mini-proteins are a class of short, structurally defined biomolecules that combine the functional specificity of larger proteins with the favourable properties of small molecules. Their stable three-dimensional conformations enable rational designs that recognize specific DNA sequences with high affinity and selectivity [2]. These features offer key advantages over traditional transcription factors and peptides, including improved stability, reduced immunogenicity, and enhanced tunability, positioning DNA-binding mini-proteins as attractive candidates for gene regulation, genome editing, molecular diagnostics, and targeted therapeutics [3].

A prominent example is OMO-103 (Omomyc for infusion), a DNA-binding mini-protein that enables direct targeting of the MYC oncogene – an established driver of tumorigenesis that has historically been difficult to inhibit [1, 4]. Omomyc forms homodimers or heterodimers with MYC or its binding partner MAX and selectively binds E-box DNA sequences (CACGTG). By displacing transcriptionally active MYC–MAX complexes from chromatin, Omomyc suppresses MYC-driven transcriptional programs essential for cancer cell growth and survival [5, 6]. This example establishes a clear mechanistic link between sequence-specific DNA binding and therapeutic activity.

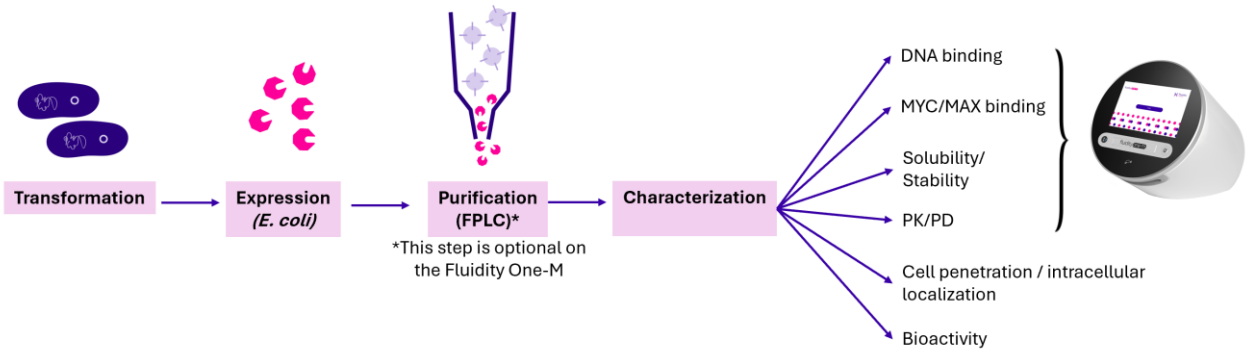

Figure 1. Typical workflow for OMO-103 variant discovery and the utility of the Fluidity One-M.

The development of Omomyc and other DNA-targeted mini-proteins relies on robust, quantitative methods to characterize protein–DNA interactions. However, commonly used surface-based techniques such as surface plasmon resonance (SPR) and enzyme-linked immunosorbent assays (ELISA) present notable limitations. DNA immobilization in SPR can perturb native binding behaviour, and in some cases, the exceptional stability of protein–DNA complexes prevent determination of dissociation rate (k_d) and dissociation constant (K_D) [7]. ELISA sensitivity can be limiting for high-affinity interactions in the nanomolar range, as measurements depend on the affinity of the detection antibody [8]. Other approaches, including circular dichroism (CD) [9] and electrophoretic mobility shift assays (EMSA) [10] are useful for validating binding interactions, but they have notable constraints related to buffer requirements and may not always deliver accurate or quantitative affinity measurements.

Microfluidic Diffusional Sizing (MDS), the measurement principle behind the Fluidity One-M, answers all three characterization needs in a single workflow. MDS measures changes in hydrodynamic radius as molecules interact in free solution, delivering quantitative affinity, stoichiometry, and complex-size data without immobilization, without purification, and in any background matrix up to 100% plasma (Table 1). In this application note, we use MDS to characterize the DNA-binding affinity, sequence specificity, and non-specific binding in plasma of three Omomyc mini-protein variants (OLS, V110, and V113), demonstrating a practical workflow for teams developing DNA-targeted therapeutics.

Capability	SPR / BLI	ELISA	CD	EMSA	MDS (Fluidity One-M)
In-solution, no immobilization	No	No	Yes	No	**Yes**
Quantitative k_d (sub-100 nM)	Limited for highly stable complexes	Detection-antibody limited	No	No	**Yes**
Works in 100% plasma or serum	No	Often No	No	No	**Yes**
Sample volume per datapoint	Variable (typically >10 µL)	Variable (50-100 µL)	High (>100 µL)	Moderate	**4 µL**
Time per binding curve	Hours	Hours to Days (with set up/optimization)	Hours (titration-based)	Hours	**< 25 Min**
Buffer restrictions	Yes	Yes	Yes	Yes	**Minimal**

Table 1. Capability comparisons of available instrumentation for DNA-targeted mini-protein characterization.

Results: Affinity and Specificity Without Immobilization & Characterizing Non-Specific Binding in 100% Plasma with F1M

MDS measures the hydrodynamic radius (R_h) of a fluorescently labelled molecule by examining its rate of diffusion in a microfluidic chamber and can be applied to determine in-solution interaction metrics by observing changes in size (upon binding). In practice, this approach involves incubating a constant concentration of fluorescent molecule, in this case canonical and non-standard DNA, with a titration of unlabeled binding partner, in this case OLS, V110 and V113. The subsequent concentration dependent change in R_h is measured and used to quantify K_D, as well as probe and complex sizes.

By using this method, differences in affinity of Omomyc variants against DNA probes were revealed. The OLS and V110 variants showed the strongest affinity against canonical DNA. As expected, all variants displayed a weaker affinity against non-standard DNA, highlighting the specificity of Omomyc and importance of the E-box sequence (CACGTG) for recognition – Table 1 and Figure 2.

		OLS	V110	V113
Canonical	R_h – free (nm)	1.84	1.70	1.69
	R_h – complex (nm)	3.73	3.36	3.36
	K_D (nM)	88.1	69.8	7490
Non-standard	R_h – free (nm)	1.48	1.50	1.50
	R_h – complex (nm)	2.71	2.75	No binding
	K_D (nM)	234	997	No binding

Table 2. Summary of R_h and K_D for DNA probes against Omomyc variants

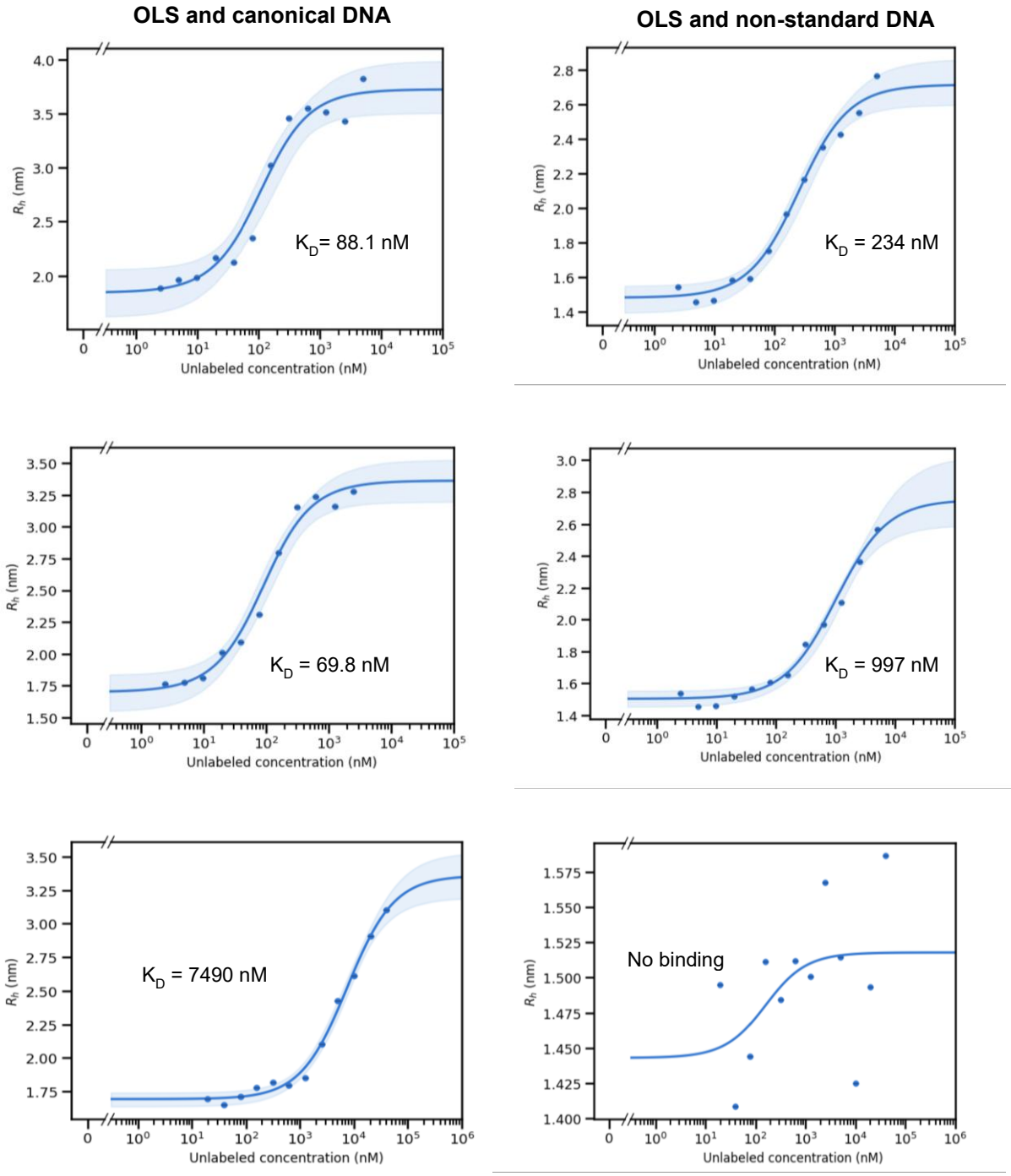

Figure 2. In-solution binding curves for labelled canonical and non-standard DNA against Omomyc variants.

Working in-solution with MDS does not require any immobilization of included molecules to a surface or chip, which brings several benefits. This includes an improved workflow with lower sample preparation and optimization time, and greater target versatility allowing analysis of molecules that are difficult to immobilize (for instance DNA). As well as permitting interactions to be examined in more native environments, MDS allows molecules and interactions to be measured across any background – including 100% plasma and other biologically relevant matrices.

Using this capability, we determined the R_h of labelled OLS, V110 and V113 in PBS-T and various concentrations of plasma (including 100%). Assuming plasma contains negligible concentrations of the target DNA binding partner, any increase in the R_h of labelled variants is attributed primarily to non-specific interactions with plasma components (Figure 3). Variants with higher affinities to canonical and non-standard DNA - V110 and OLS -displayed a larger change in R_h with increasing percentages of plasma, albeit V110 appeared to have a comparatively lower affinity to non-specific components.

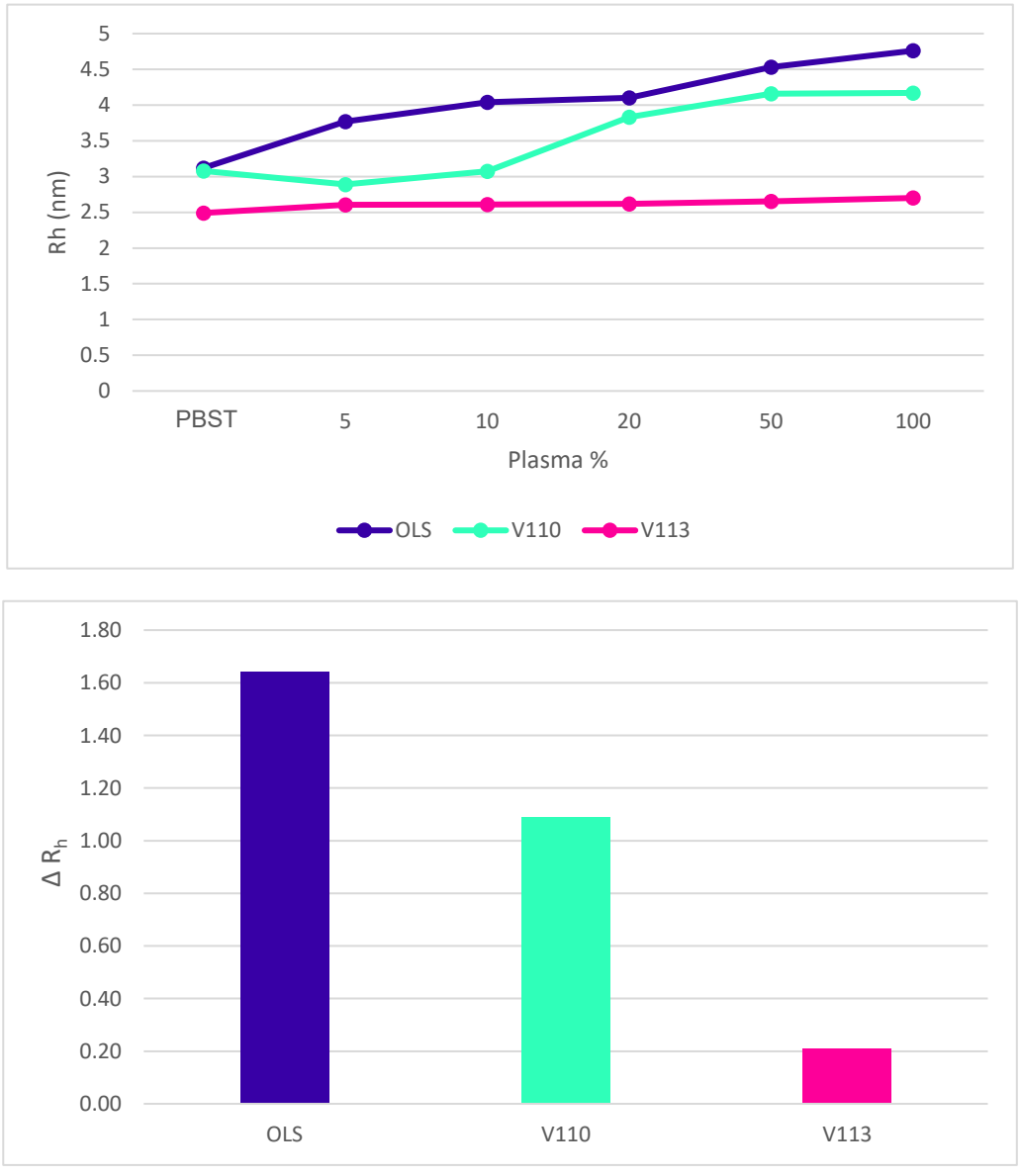

Figure 3. Average R_h of OLS, V110 and V113 in various plasma concentrations up to 100% (above). Change in R_h between PBST and 100% plasma for each variant (below). A greater change in R_h is indicative of higher non-specific binding to plasma components.

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Conclusion: Complete In-Solution Characterization on One Platform

Peptomyc's Omomyc program (clinical-stage compound OMO-103 demonstrated safety and signs of activity in a Phase 1 trial published in *Nature Medicine*) illustrates a challenge shared by teams developing DNA-targeted mini-proteins: characterization workflows are fragmented across multiple instruments, none of which operate in physiologically relevant matrices. The Fluidity One-M overcomes that challenge.

For teams developing DNA-targeted mini-proteins, peptides, or other sequence-specific therapeutics, MDS answers the three characterization questions that matter (e.g. variant affinity, target specificity, and non-specific binding in physiological matrices) on a single platform, without rebuilding the assay for each stage. The Fluidity One-M requires 4 µL per datapoint, delivers results in under 25 minutes, and works in backgrounds from buffer through 100% plasma. From early variant screening to translational readiness, the workflow is the same.

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Methods

System: Fluidity One-M

Labelling:

- Omomyc variants (OLS, V110 and V113): Alexa Fluor 488-C4-maleimide
- DNA: fluorescein – thymine modified nucleic acid-647

Buffer: PBST (0.1% polysorbate) or mouse plasma

Sample volume: 4 µL per datapoint

Time: <25 minutes per binding curve, <30 minutes per non-specific binding assay.

Measuring affinity of fluorescently labelled DNA and Omomyc variants

Canonical and non-standard DNA probes were purchased from Integrated DNA Technology and labelled on their 5' end (forward only) with a Fluorescein - thymine modified nucleic acid-647. Labelled canonical and non-standard DNA probes were diluted in PBST to a final concentration of 40 nM and incubated with a titration of Omomyc variants across a concentration range between 0.002 and 5 µM (OLS and V110) and 0.02 and 40 µM (V113). Samples were incubated for at least 30 minutes at room temperature. R_h measurements were run on viscosity setting 1 on size setting 1 (1 – 4.7 nm), using the red 647 detection settings.

Measuring non-specific binding of Omomyc variants and plasma components

Omomyc variants – OLS, V110 and V113 – were labelled with Alexa Fluor 488 – C4 – maleimide which reacted to the sole Cys residue present near the C-terminus of the proteins. Labelled variants were diluted to a final concentration of 1 µM in phosphate buffered saline-Tween (PBS-T) and 5%, 10%, 20%, 50% and 100% plasma from C57BL/6 mice, and R_h was measured in triplicates. Samples were incubated for at least 30 minutes at room temperature. R_h measurements were run on viscosity setting 1 for PBS-T, 5% and 10% plasma conditions and viscosity setting 2 for 20%, 50% and 100% plasma conditions. All measurements were made on size setting 1 (1 – 4.7 nm), using the green 488 detection settings.

About us

It’s not just the proteins that make life, it’s the interactions among them. Here at Fluidic Sciences, we make protein interaction analysis easy and robust by developing transformative in-solution technologies and accessible instruments that help scientists quickly and accurately understand how proteins truly interact.

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