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# Modeling the O26 polysaccharide capsule as an antigen-accessibility barrier: a reproducible in silico framework for antibody-mediated anti-adhesion

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# Abstract

The O26 O-antigen capsule has been implicated in immune evasion, while anti-O26 polysaccharide antibodies can restore immune recognition and inhibit epithelial adhesion. However, the published evidence does not contain a quantitative anti-adhesion dose-response series from which a capsular effect size can be estimated. We therefore developed a reproducible in silico framework that represents functional antigen accessibility by a dimensionless parameter (α) within a normalized Hill response. The model defines a Capsular Accessibility Penalty (CAP) as the fold-shift in the antibody activity required for half-maximal inhibition relative to full accessibility. Parameter-space exploration used α = 0.20-1.00 and Hill coefficients n = 0.80-2.00, with 100,000 seeded simulations for sensitivity analysis. Analytically, CAP = 1/α; thus accessibility controls horizontal displacement of the response curve, whereas n primarily controls transition width. CAP was perfectly inversely monotonic with α (Spearman ρ=-1.000) and essentially independent of n (ρ = 0.001). By contrast, response at a single antibody activity depended on both α and n, demonstrating non-identifiability from a fixed-condition assay. The framework converts a qualitative capsule-accessibility hypothesis into a transparent quantitative model while preserving the distinction between published experimental evidence and exploratory computational inference.


# Introduction

Diarrheagenic *Escherichia coli* comprise diverse pathotypes that use distinct virulence programs to colonize the intestinal mucosa, damage epithelial cells, and evade host immunity [1, 2]. Serogroup O26 is particularly important because it includes enteropathogenic *E. coli* (EPEC) and Shiga toxin-producing/enterohemorrhagic *E. coli* (STEC/EHEC) lineages and has been repeatedly associated with severe human disease, including hemorrhagic colitis and hemolytic uremic syndrome [3–5]. Although the molecular epidemiology and protein-mediated virulence of O26 have been extensively investigated, quantitative relationships between surface-polysaccharide accessibility and antibody-mediated anti-adhesion remain less developed.

In O26, a previous experimental study showed that capsulated EPEC O26:H11 resisted complement-mediated immune mechanisms more effectively than the non-capsulated comparator and that anti-O26 polysaccharide antibodies restored immune recognition and inhibited adhesion of capsulated O26 EPEC to human epithelial cells [6]. The outer surface context is relevant because *E. coli* contains lipopolysaccharide, O antigen, and, in some lineages, polysaccharide capsules that can alter exposure of underlying or chemically related epitopes. Capsules can reduce complement deposition, opsonization, and phagocytic clearance [7–9], and an O-antigen capsule can modulate pathogenesis by changing how immune effectors access the bacterial surface [10]. Importantly, the published O26 anti-adhesion experiment used a fixed antibody condition and demonstrated the phenomenon qualitatively rather than as a quantitative dose-response series.

Antibody-mediated interference with epithelial adhesion is biologically plausible beyond O26. Antibodies directed against intimin can reduce EPEC attachment and actin rearrangement [11, 12], and antibodies

against O111 lipopolysaccharide were reported to recognize multiple diarrheagenic *E. coli* categories and inhibit adhesion to human epithelial cells [13]. These observations support anti-adhesion as a functional outcome of humoral immunity, but they do not by themselves establish how much additional antibody activity would be required when a surface capsule decreases antigen accessibility.

This study addresses that gap by treating capsular masking as a quantitative accessibility problem rather than by reconstructing unavailable historical measurements. We formulated a normalized Hill-response model in which a dimensionless accessibility factor, α, modifies effective antibody activity. From this model we derived a Capsular Accessibility Penalty (CAP), defined as the fold increase in normalized antibody activity required to reach the same half-maximal response relative to full accessibility. We then evaluated the model across a prespecified exploratory parameter space, quantified sensitivity and response-width behavior, and examined parameter identifiability under fixed-condition versus dose-response observations. The aim was not to estimate a biological CAP for O26 from insufficient data, but to create a reproducible framework that converts the experimentally supported accessibility hypothesis into explicit, testable quantitative predictions.

## Materials and methods

# Study design and evidence boundary

This work is a secondary computational analysis anchored to previously published biological evidence. No new bacterial culture, cell-culture experiment, animal procedure, human-participant procedure, or biological intervention was performed. The principal O26 anchor was the 2024 study by Lemos et al. [6], which documented an O-antigen capsule in EPEC O26:H11, capsule-associated impairment of complement-related immune recognition, reversal of part of that immune evasion by anti-O26 polysaccharide antibodies, and inhibition of epithelial adhesion by those antibodies. Because the published anti-adhesion assay was conducted at a fixed antibody condition and did not provide a numerical concentration-response series, no O26-specific half-response parameter was fitted from that experiment.

The analysis also used prior literature as mechanistic context: classical work showing that *E. coli* capsules can impede complement fixation and phagocytosis [7, 8], broader discussion of complement evasion [9], evidence that an O-antigen capsule can alter surface-dependent pathogenesis [10], and published demonstrations that antibodies can inhibit epithelial adhesion through protein or polysaccharide targets [11–13]. These sources were used to define a biologically plausible model structure, not to manufacture missing O26 measurements.

## Model formulation

Let C denote effective anti-O26 antibody activity and Kref the half-response scale under a reference condition of maximal functional accessibility. The normalized activity is x = C/Kref. Functional antigen

accessibility is represented by α, where $0 < \alpha \leq 1$; $\alpha = 1$ is the reference state and $\alpha < 1$ represents reduced effective accessibility. The normalized inhibition response was defined using a Hill function [14]: $I(x;\alpha,n)=(\alpha x)^n/[1+(\alpha x)^n]$, where $n > 0$ is the Hill coefficient and the maximum response is normalized to 1. In this formulation, α is an abstract accessibility factor and is not equated to a measured capsule thickness, epitope density, or a specific strain genotype.

For any target response fraction p between 0 and 1, the normalized activity required to reach p is $Cp=(1/\alpha)[p/(1-p)]^{(1/n)}$. At $p = 0.5$, $C50 = 1/\alpha$. We therefore define $CAP=C50(\alpha)/C50(\alpha = 1) = 1/\alpha$. CAP is a model-derived fold shift in required effective antibody activity. A CAP of 2, for example, means that a condition represented by $\alpha = 0.5$ requires twice the normalized effective activity to reach the same half-maximal response as the reference condition; it does not mean that O26 has been empirically measured to have a twofold penalty.

## Parameter-space design and computational analysis

The computational design intentionally explored a broad normalized space rather than assigning empirical priors that the available O26 data cannot support. Accessibility α was sampled uniformly from 0.20 to 1.00 and n from 0.80 to 2.00. These ranges are design-space boundaries only. They are not biological probability distributions and their quantiles must not be interpreted as confidence or credible intervals for O26. The maximum normalized response was fixed at 1 to isolate curve geometry.

A seeded Monte Carlo exploration of 100,000 α-n combinations was performed. CAP, inhibition at normalized activities $x = 1$ and $x = 1.5$, and rank-based sensitivities were calculated. Spearman correlations were used to summarize monotonic dependence between sampled parameters and outputs. Threshold metrics C10, C50, and C90 were calculated analytically, and transition width was expressed as $\log10(C90/C10)$. Because CAP has a closed-form solution, the simulation was used primarily to verify computational implementation and to characterize how fixed-activity responses behave when both α and n vary.

## Identifiability analysis

Identifiability was evaluated conceptually and algebraically. A full multi-point dose-response curve can, in principle, inform both horizontal displacement and slope, whereas a single fixed-condition response cannot uniquely separate α, n, and the response scale. Because the published O26 anti-adhesion experiment provides only a fixed antibody condition rather than a numerical dose-response series, α, n, Kref, and CAP were not fitted to that experiment.

## Software, reproducibility, and AI-assisted workflow

Computations were implemented in Python 3.13.5 using NumPy 2.3.5, pandas 2.2.3, SciPy 1.17.0, and Matplotlib 3.10.8. The random seed was fixed at 20261024. The analysis code, scenario table, curve grid, sensitivity summary, and threshold metrics are preserved as supplementary computational files. Generative AI tools were used during project structuring, drafting, and workflow assistance. All source selection, equations, code, numerical outputs, interpretations, and final manuscript decisions were reviewed by the human authors, who assume full responsibility for the work.

Table 1
Model quantities and computational design

| Quantity | Definition | Value/range | Interpretation |
|---|---|---|---|
| x | Normalized effective antibody activity, C/Kref | Approx. 0.05-20 in plotted grid | Dimensionless model input |
| α | Functional antigen accessibility | 0.20-1.00 | Exploratory design space; not an empirical prior |
| n | Hill coefficient | 0.80-2.00 | Exploratory response-slope range |
| Imax | Maximum normalized inhibition | 1 | Normalization constant |
| CAP | C50(α)/C50(1) = 1/α | Derived | Model-level accessibility penalty |
| N | Monte Carlo samples | 100,000 | Global design-space exploration |
| Seed | Random-number seed | 20261024 | Reproducibility |

## Results

# Structural relationship between accessibility and half-response shift

The model yields an exact relationship between functional accessibility and half-response position: C50 = 1/α and CAP = 1/α. Reducing α therefore produces a horizontal right-shift in the normalized inhibition curve without requiring a change in maximal response. Illustrative scenarios show CAP values of 1.25, 1.49, 2.00, 2.50, 4.00, and 5.00 for α values of 0.80, 0.67, 0.50, 0.40, 0.25, and 0.20, respectively (Table 2). These are deterministic scenario outputs, not estimates of O26 biology.

Figure 1 shows normalized dose-response curves across representative accessibility values at n = 1.2. Decreasing α displaces the curve toward higher normalized antibody activity. Changing n instead alters the sharpness of the transition. This separation is also visible analytically because the ratio C90/C10 depends on n but not on α.

Table 2
Illustrative accessibility scenarios and deterministic half-response penalty

| Functional accessibility α | CAP = 1/α |
|---|---|
| 1.00 | 1.00 |
| 0.80 | 1.25 |
| 0.67 | 1.49 |
| 0.50 | 2.00 |
| 0.40 | 2.50 |
| 0.25 | 4.00 |
| 0.20 | 5.00 |

## Threshold behavior and sensitivity

For n = 0.8, 1.2, and 2.0, the log10 transition widths C90/C10 were 2.386, 1.590, and 0.954, respectively, independent of α. Thus, within this multiplicative-accessibility model, accessibility determines the horizontal penalty while n determines how rapidly the response changes around the midpoint.

Across 100,000 exploratory α-n combinations, CAP had a median of 1.663 and a 5th-95th percentile range of 1.041–4.168 solely because α was sampled uniformly between 0.20 and 1.00. These values describe the sampled design space and are not biological uncertainty intervals. Spearman correlation between α and CAP was − 1.000, whereas correlation between n and CAP was 0.001. At a fixed normalized activity x = 1, response correlated strongly with α (ρ = 0.957) but also depended on n (ρ=-0.264). The latter result demonstrates that a response measured at only one antibody condition cannot uniquely identify the accessibility penalty.

Table 3
Global design-space sensitivity summary

| Metric | Result |
|---|---|
| Monte Carlo samples | 100,000 |
| CAP median (design space) | 1.663 |
| CAP 5th-95th percentile (design space) | 1.041–4.168 |
| Spearman ρ: α vs CAP | -1.000 |
| Spearman ρ: n vs CAP | 0.001 |
| Spearman ρ: α vs response at x = 1 | 0.957 |
| Spearman ρ: n vs response at x = 1 | -0.264 |

# Identifiability of a capsular accessibility penalty

The published O26 anti-adhesion result provides qualitative support for antibody-mediated inhibition but does not identify α, n, Kref, or CAP because it does not contain a numerical dose-response series. Similarly, the published comparison between capsulated EPEC O26:H11 and a non-capsulated EHEC O26:H11 comparator is not isogenic, so strain-level differences cannot be assigned quantitatively to the capsule alone. The present study therefore reports only structural and design-space properties of the model and does not assign an empirical CAP to O26.

## Discussion

This study converts a biologically supported but previously qualitative proposition into an explicit quantitative framework: reduced functional access to an O26 surface antigen can be represented as a multiplicative penalty on effective antibody activity. The key contribution is not a new estimate of capsule thickness or antibody potency, but a separation of three concepts that are easily conflated in interpretation of a fixed-condition assay: accessibility (α), response slope (n), and the amount of antibody activity required to reach a defined functional threshold. The derived CAP metric provides a compact way to express the horizontal consequence of accessibility loss while keeping that consequence conditional on model assumptions.

The model is biologically aligned with evidence that polysaccharide capsules can shield bacterial surfaces from complement and phagocytes [7–9] and with observations that O-antigen capsules can modulate surface-dependent interactions [10]. In the O26 system, the experimental anchor is particularly relevant because anti-O26 polysaccharide antibodies were able to overcome part of capsule-associated immune evasion and inhibit epithelial adhesion [6]. The present framework does not reinterpret those observations as a measured concentration-response curve; instead, it asks what dose-response

geometry would be expected if the capsule acts primarily by reducing the fraction of antibody activity that effectively reaches or engages a functional surface target.

The distinction between α and n is useful for experimental interpretation. In the simple model, accessibility changes C50 without changing the logarithmic C90/C10 width, whereas n changes width without determining CAP. A future quantitative data set that shows a predominantly parallel shift would therefore be compatible with a multiplicative accessibility mechanism, while a major change in slope would suggest that additional processes are required. Those processes could include heterogeneous epitope exposure, multiple functional antibody populations, aggregation, or downstream nonlinearities. The current model intentionally does not add such mechanisms because the available published data do not identify them.

The identifiability analysis is equally important. A single antibody condition can show that inhibition occurs, but the observed response is compatible with many combinations of accessibility, slope, and scale. Fixed-condition anti-adhesion evidence therefore should not be converted retrospectively into a unique C50 or fold-shift. Keeping the model uncalibrated at this stage makes the boundary between published observation and computational hypothesis explicit and prevents false precision.

The framework also has relevance for O-antigen vaccine development. O-specific polysaccharide conjugates have demonstrated immunogenicity in human studies [15–18], and modern bioconjugate and glycoconjugate platforms continue to expand the feasibility of multivalent *E. coli* vaccines [19–22]. Antibody concentration alone, however, does not necessarily capture functional access to a bacterial target. For a surface antigen that can exist in different architectural contexts, a functional assay can be interpreted more effectively when potency, accessibility, and curve shape are considered separately. CAP is proposed as a model-level descriptor for that purpose, not as a replacement for measured neutralization parameters.

Several limitations define the appropriate scope of the present study. First, α is not experimentally measured in O26 and the sampled α range is deliberately exploratory. Second, n is also not estimated from O26 anti-adhesion data. Third, the 2024 O26 study compared a capsulated EPEC strain with a non-capsulated EHEC comparator rather than an isogenic capsule pair; therefore the biological difference between those strains cannot be reduced to capsule status. Fourth, the published anti-adhesion experiment used a fixed antibody condition and qualitative microscopy, which prevents empirical calibration of CAP. Finally, the multiplicative Hill formulation assumes that accessibility acts upstream of a common sigmoidal response and does not model agglutination, epitope heterogeneity, antibody subclass effects, or multiple adhesion pathways.

These limitations determine the next evidentiary requirement without requiring reconstruction of lost data: biological calibration would need quantitative anti-adhesion measurements across multiple antibody activities under appropriately matched bacterial conditions, together with sufficient replication to estimate uncertainty. Until such data exist, the defensible conclusion is structural. If capsular masking can be represented as a reduction in functional accessibility, then the model predicts a proportional

horizontal penalty CAP = 1/α, while slope effects remain distinguishable through response-width metrics. This prediction is directly testable and provides a transparent bridge between the published O26 phenotype and future quantitative validation.

## Conclusions

A normalized Hill framework can represent the O26 capsule-accessibility hypothesis without converting unavailable historical measurements into new evidence. The model defines CAP = 1/α as the fold increase in effective antibody activity required for half-maximal anti-adhesion when functional accessibility is reduced, while the Hill coefficient primarily governs response width. Sensitivity and identifiability analyses show why a fixed antibody condition cannot determine a unique capsular penalty. The published O26 evidence therefore supports the biological premise that anti-O26 antibodies can overcome capsule-associated immune evasion and inhibit epithelial adhesion, but it does not support a specific empirical CAP or D50 shift. The present computational framework provides a reproducible, explicitly bounded hypothesis-generation tool for interpreting future quantitative anti-adhesion data and for integrating antigen accessibility into functional evaluation of O-antigen-directed vaccine responses.

## Declarations

# Competing interests

The authors declare that they have no competing financial or non-financial interests.

# Ethics approval

Not applicable to the present analysis. No new human participants, animals, biological samples, or interventions were used; the biological evidence was obtained from previously published literature.

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This research did not receive funding.

# Author Contribution

T.J.S.L.: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Validation, Writing – original draft, Writing – review & editing. M.A.Z.: Methodology, Project administration, Visualization, Writing – review & editing.

# Data Availability

All data generated or analyzed during this study are included in this manuscript and its supplementary information files. The computational code, parameter definitions, input tables, and model outputs required to reproduce the analyses are provided in Online Resource 1. The published experimental evidence used to motivate and contextualize the model is available in the cited literature.

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# Figures

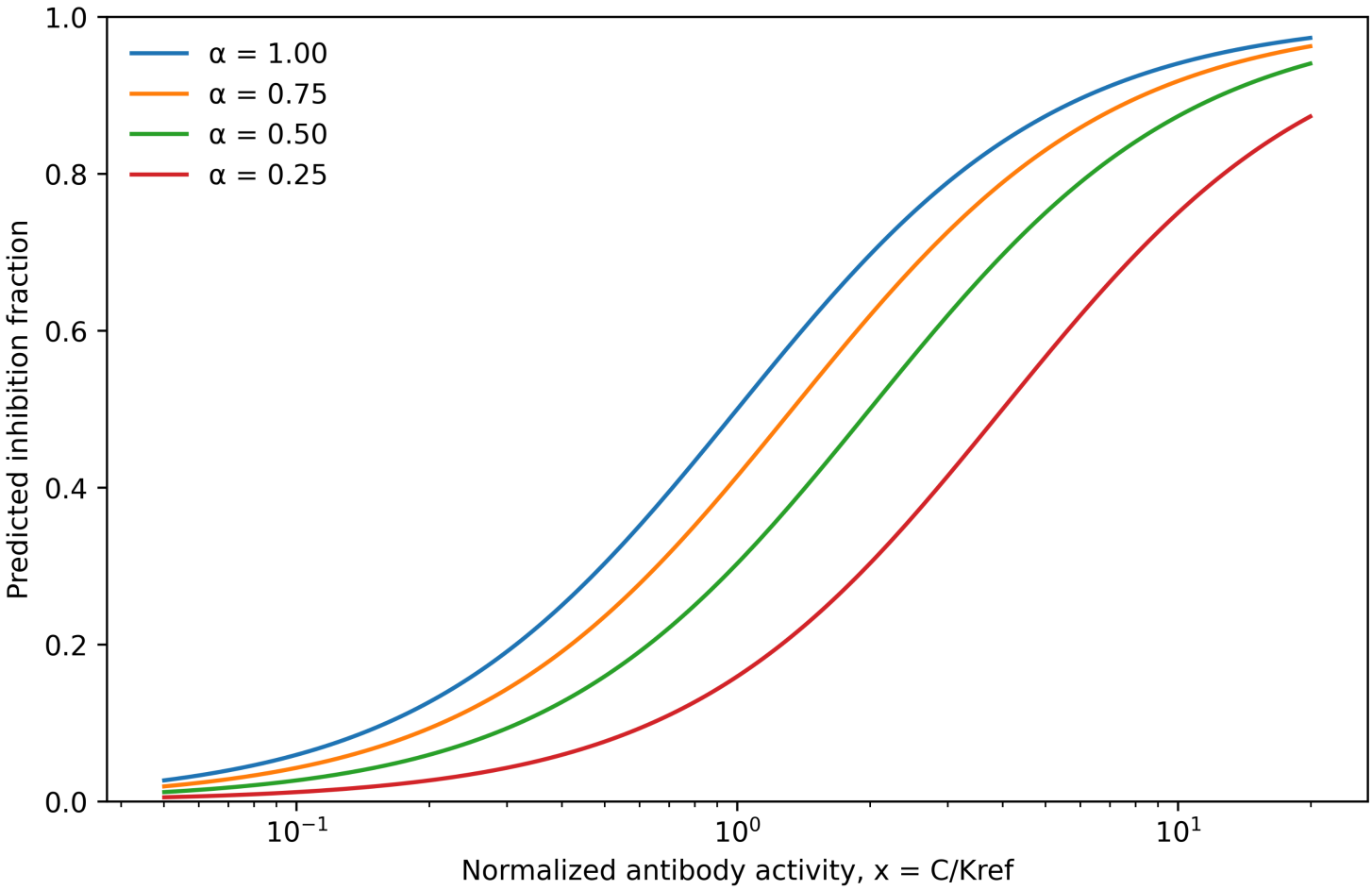



**Figure 1**

Normalized Hill responses at n=1.2 for representative accessibility values. Lower α produces a right-shift in the activity-response relationship. Curves are dimensionless exploratory outputs and are not calibrated estimates for O26

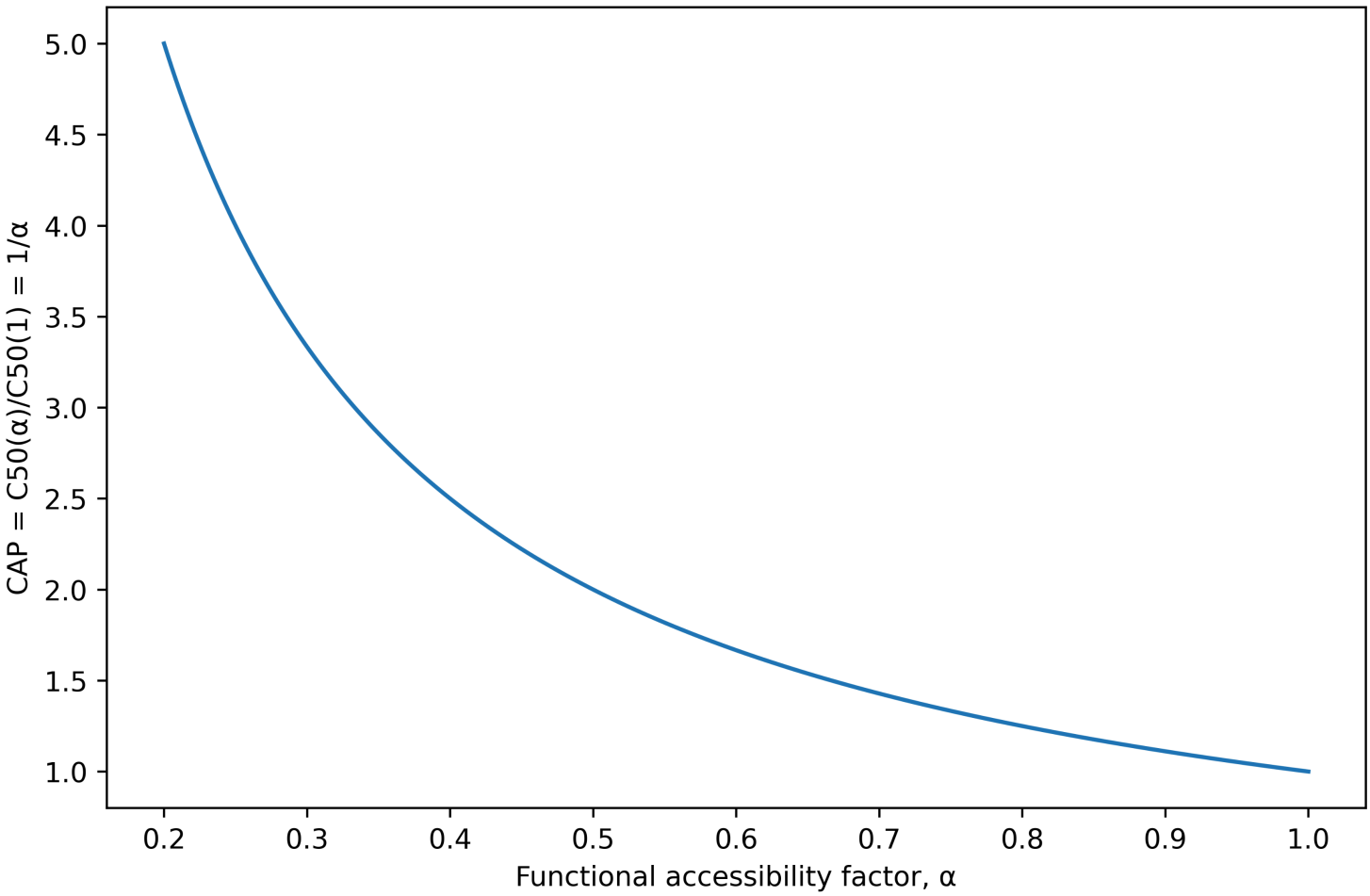



**Figure 2**

Structural relationship between functional accessibility and the Capsular Accessibility Penalty (CAP). CAP rises as 1/α by model definition; the curve describes model geometry, not an empirically estimated distribution for O26

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 03O26BJMOnlineResource1.zip